Mr. Ashby

TECHNICAL BULLETIN NO. 131 AUGUST, 1927

NEW YORK STATE AGRICULTURAL EXPERIMENT STATION

Geneva, N. Y.

IN COOPERATION WITH THE
DEPARTMENT OF DAIRY INDUSTRY,
VERMONT AGRICULTURAL EXPERIMENT STATION,
BURLINGTON, VT.

THERMOPHILIC AND THERMODURIC MICRO-ORGANISMS WITH SPECIAL REFERENCE TO SPECIES ISOLATED FROM MILK

III. DESCRIPTION OF THE NON-SPORE-FORMING, THERMODURIC ORGANISMS ISOLATED

A. H. ROBERTSON

PUBLISHED BY THE STATION
UNDER AUTHORITY OF CORNELL UNIVERSITY

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TECHNICAL BULLETIN No. 131

THERMOPHILIC AND THERMODURIC MICRO-ORGANISMS WITH SPECIAL REFERENCE TO SPECIES ISOLATED FROM MILK

III. DESCRIPTION OF THE NON-SPORE-FORMING, THERMODURIC ORGANISMS ISOLATED[1]

A. H. ROBERTSON[2]

ABSTRACT

The present study is an outgrowth of an attempt to determine one or more of the bacterial species which may cause the so-called "pin-point" colonies on pasteurized milk plates.

Cultures of non-spore-forming, thermoduric bacteria have been secured from the following sources: (1) Pasteurized milk plates containing "pin-point" colonies from several cities in this country, (2) milk which had been incubated at pasteurizing temperatures for several hours, (3) milking machines which had been improperly washed and sterilized, and (4) accidental contaminations.

Cultures in which 90 per cent or more of the cells survived pasteurization were retained for further study. These cultures belong to the following five species named in the order of their resistance to heat: *Microbacterium lacticum* Orla-Jensen, *Sarcina lutea* Schröter, *Streptococcus thermophilus* Orla-Jensen, *Sarcina rosea* Schröter, and *Micrococcus conglomeratus* Migula. *Microbacterium lacticum* and *Streptococcus thermophilus* may form "pin-point" colonies on agar plates.

The presence of the above species in market milk is not regarded as dangerous to health.

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[1]Portion of a thesis submitted to the Graduate School at Cornell University in partial fulfillment of the requirements for the degree of doctor of philosophy. The entire contribution appears in four parts, two of which are Technical Bulletins Nos. 130 and 131 of the New York State Agricultural Experiment Station and two Bulletins Nos. 274 and 275 of the Vermont Agricultural Experiment Station. The author acknowledges the many helpful suggestions offered during the course of the present investigation by Dr. R. S. Breed of the New York Station and by Dr. H. B. Ellenberger and Dr. B. F. Lutman of the Vermont Station.

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INTRODUCTION

A large part of the milk consumed in the cities of this country is pasteurized. Since the World War the unusual sporadic appearance of large numbers of small, punctiform, white bacterial colonies has been observed on agar plates prepared from pasteurized milk in some of these cities. At first these were attributed to unsanitary practices in the pasteurizing plants, but controlled experiments revealed that certain bacteria grew during pasteurization. During pasteurization, several samples of the pasteurized milk might be taken at intervals and the bacterial counts of these samples would be normal at first, i.e., under 30,000 colonies per cc, when suddenly there would be an increase to 200,000 per cc, or even more. Dotterrer (1923)[3] compared samples taken in this way with a series of samples taken approximately 30 minutes before the pasteurized sample was secured. The counts of the raw milk samples indicated that the increase was not necessarily associated with an increase in the bacterial content of the milk in the supply tank.

The flora in question appears and disappears sporadically in some plants and is an addition to the flora of pasteurized milk which has been earlier observed. Since many cities have ordinances limiting the bacterial content of both raw and pasteurized milk, a further study of the thermophilic and thermoduric, non-spore-forming bacteria of pasteurized milk has been made. In this report, a 90 per cent survival as determined by the agar plate method was required of any bacterial species before it was regarded as heat-resistant. Therefore the species which are given extended study had a "majority thermal death point" above the pasteurization temperatures used.

HISTORICAL

The literature on the presence of non-spore-forming bacteria in pasteurized milk is not extensive. The related work on the survival of bacteria when exposed to heat can not properly be omitted. Probably Ayers and Johnson (1913) have contributed the most comprehensive study of pasteurization methods and temperatures with special reference to their influence upon the flora observed. The authors have observed some of the fermentative powers of a large number of cultures and divide the species into five groups, the weak acid-forming types, the acid and coagulating, the inert, the al-

[3]Refers to Literature Cited, page 54.

kali-forming, and the peptonizing types, according to their action in litmus milk. Pasteurization increased the percentage of the acid-forming group in milk, while the other groups decreased. The average percentage of lactic acid bacteria in raw milk which survived pasteurization for 30 minutes at 62.8°C ranged from 1.27 to 4.55 in the various grades of milk. Pasteurization at temperatures above 76.7°C for three minutes largely destroys the acid-forming bacteria and the spores of the peptonizing types remain alive. For the purpose of comparison, the types surviving 62.8°C for 30 minutes will be discussed later (page 22) in association with the isolations made in the present investigation.

Sternberg (1887) was among the first to observe the thermal death points of some of the pathogenic bacteria. Some of the species of micrococci were able to resist 62°C for 10 minutes and 60° C for 30 minutes. Hauser (1887) also found certain of the micrococci to be more resistant to heat than others. Adametz (1890) found certain species of sarcinae in both raw and sterilized milk.[4] Van Geuns (1889) heated pure cultures of white and yellow micrococci, white, green, and gray short rods, and white, gray, orange, red, and violet non-spore-forming long rods at 80° C for 90 seconds. After heating the cultures were cooled to 12° C. Two cultures of white micrococci, a translucent, gray, non-liquefying, short rod and a white liquefying, long rod, survived. He found a chain-forming micrococcus in slime which survived 70° C for 90 seconds. Van Geuns (1889) and de Man (1893) reported that the non-spore-forming pathogenic species were usually killed by exposure to 60° C for 90 seconds.

Dyar (1895) isolated a yellow, non-liquefying, nitrate-reducing micrococcus (probably a sarcina) from a jar of sterilized milk which was heavily seeded with this species. After growth in milk for six days, the milk was coagulated on boiling. Stubenrath (1897) found the sarcinae to be unusually resistant to heat. Marshall (1897) was perhaps the first to report the characteristic lactic acid fermentation in pasteurized milk similar to that occurring in raw milk. Weiss (1899) isolated three lactic acid species from mash, the optimum growth temperatures of which are between 40° and 45° C.

Smith (1899) showed that the surface layer of pasteurized milk has a protective action on the tubercle bacilli. Hashimoto (1901) described two lactic acid, coccus forms (a micrococcus and a strepto-

[4]Term used to signify practical sterilization.

coccus) which were isolated from sterilized milk. Russell and Hastings (1901, 1902a) confirmed the observations of Smith (1899) by proving that bacteria in the scalded layer surface pellicle of milk heated in contact with air survive, while bacteria in the depths of the liquid are killed. Both the cooling effect of evaporation and the nature of the scalded layer itself produce the increased resistance of bacteria in milk pasteurized in contact with the air. The scalded layer has a lower moisture content and under these conditions heat rigor is retarded.

Russell and Hastings (1902b) describe a Gram-positive, non-liquefying, bright lemon-yellow micrococcus, the thermal death point of which is 76° C in milk. The authors assume that a different kind of protoplasm explains the unusual heat resistance of this micrococcus. Freudenreich and Thöni (1904) studied a number of highly heat-resistant lactic acid bacteria. They reported that *Bacterium lactis acidi* Leichmann (1896) survived 60° C for 30 minutes, 65° C for 15 minutes, and 70° C for 5 minutes. Rogers (1905) found lactic acid bacteria in milk pasteurized at 85° C by the flash method and held at 20° C, which occasionally, after 24 hours, completely inhibited the peptonizing bacteria. Henneberg (1906), attempting to prevent the growth of undesirable yeasts and bacteria in malt, found that a culture of the lactic acid bacterium, *Bacillus delbrücki* Leichmann (1896), was killed at 65° C in five minutes and at 72.5° C in five minutes when in a concentrated mass.

De Jong and de Graaff (1906) isolated a strain of *Escherichia coli* Castellani and Chalmers which withstood 85°C. Frequently it survived 65° to 66°C for 30 minutes. The culture was killed more easily in bouillon than in milk. On one occasion this culture survived 70° to 72°C for 30 minutes. The authors, therefore, recommend a temperature of 70°C for a period of 30 minutes to kill pathogens. Zelenski (1906) found a strain of *Escherichia coli* which survived unusually high temperatures. Gage and Stoughton (1906) showed that the majority thermal death point of *Escherichia coli* was 50° to 60°C. Attempts to reproduce and develop a progeny with greater heat resistance from colonies that survive the higher temperatures failed. Adelloff (1907) reported that some of the lactic acid bacteria survive pasteurization temperatures.

Wolff (1908) pasteurized milk at 70°C and found that some of the lactic acid bacteria survived. The percentage survival depended to some degree on the amount of acid present which aided in the for-

mation of a protective covering of casein and albumin about the cells so that they could not only resist the higher temperatures but also carry other organisms along with them.

Eijkmann (1908) first discussed the shape of the rate-of-killing curve for bacterial cultures when exposed to heat. The curve assumes a reversed "S" appearance, showing that a few cells are killed at first, then a majority are killed suddenly, followed by a few possessing an unusually high heat resistance. Rosenau (1908) confirmed the earlier work of Sternberg (1887), Van Geuns (1889), and de Man (1893) in which they have studied the thermal death points of some of the pathogens.

Greig-Smith (1909) and Downs (1925) associated *Micrococcus albus* Buchanan with the coagulation of canned condensed milk. Ayers and Johnson (1910) proved that lactic acid bacteria of high thermal death point are frequently found in milk. In milk pasteurized at 60°C for 30 minutes, the percentage of acid-forming colonies that survived (as determined by plating) ranged from 0.001 to 18.91, the average being 4.8 per cent of the total acid colonies. As the temperature used is increased, the percentage surviving is gradually decreased so that when 65.6°C is employed for 30 minutes the average survival is only 0.74 per cent of the total number. The thermal death point of one lactic acid organism which was isolated from milk is 74.4°C in broth and 75.6°C in milk when heated in Sternberg bulbs for 30 minutes. When heated ten minutes the thermal death point is 77.8°C. In pasteurized milk and in clean raw milk, the relative proportions of the groups of bacteria, i.e., acid forming, alkali or inert forms, and the peptonizing types, are approximately the same. The alkali or inert forms are in the majority in both cases. They increase in proportion during the first day and then gradually decrease until the fifth day when the proportion is lower than that of the lactic acid group. The acid group constitutes between 20 and 30 per cent of the total and after a decrease during the first 20 hours gradually increases to form the largest proportion on the sixth day. The proportion of the peptonizing types increases during the first 24 hours and then gradually decreases.

Lingelsheim (1912) reported that *Streptococcus brevis* withstood 65°C for 20 minutes, 70°C for 10 minutes, and 80°C for 5 minutes. Ayers and Johnson (1915) discovered that the thermal death point of 174 cultures of the colon bacillus isolated from cow feces, milk, cream, human feces, flies, and cheese varied considerably

when heated in milk for 30 minutes under conditions similar to pasteurization. At 60°C, the lowest pasteurization temperature used, 95 cultures (54.59 per cent) survived and at 62.8°C, 12 (6.89 per cent) survived. One culture was not killed at 65.6° C on one occasion only. On all subsequent trials it was killed at this temperature. All cultures had low majority death points and survived on account of a few cells only. Holman (1914) found that certain albuminous liquids possessed a protective action for bacteria against heat. Gorini (1915) believed that the resistance of non-spore-forming bacteria depended on the formation of a protective coating of casein around the bacteria, the latter being responsible for the coating. Peiser (1915) pointed out that the protective action given to the suspended lactic acid bacteria in separated milk by the casein and coagulated albumin raises their thermal death point 2.5°C over that in bouillon and that the protein and fat of whole milk raises it 5.0°C. Weigmann, Wolff, Trensch, and Steffen (1916) report that the lactic acid bacteria are in larger proportions in the pasteurized milk than in the same milk before pasteurization. Souring is retarded in the pasteurized milk, altho the proportion of the acid bacteria is greater.

Patzschke (1916) isolated a *Streptococcus lactis thermophilus* which was killed after a temperature of 85°C had been maintained for from 10 to 20 minutes. Brown and Peiser (1916) concluded that casein and fat in milk offered some protection to bacteria when the latter are subjected to high temperatures. Ayers, Johnson, and Davis (1918), working with 35 strains of pathogenic streptococci, found 8 strains which survived 140°C for 30 minutes. According to their other cultural characters these eight strains would not be classed as pathogenic. As the pH varied from neutrality, the thermal death rate increased. Twiss (1920) reported that some of the members of the para-typhoid group survived pasteurization temperatures. Krumweide and Noble (1921) pointed out that some error had been introduced into Twiss's work as they were unable to obtain the same results. Gorini (1921) reported that the non-spore-forming lactic acid bacteria are killed if exposed for 15 minutes at 60° to 80° C. One case is mentioned where the thermal death point was 100° C. In the case of the acid-rennet-forming coccus, it is completely enveloped in a covering of casein.

Orla-Jensen (1919, 1921) discussed extensively the thermal resistance and the growth relationships to temperature of the lactic acid

bacteria, among which are *Streptococcus cremoris, S. thermophilus, S. lactis, Microbacterium lacticum, Thermobacterium bulgaricum, T. helveticum,* and certain sarcinae. Göbel (1923) found that the resistance to heat of the individual cell was greater in a dilute suspension than in a more concentrated suspension of the cells. Bleyer (1923) succeeded in recovering cultures of streptococci from the liver, spleen, and heart-blood of rats after their death. Prior to inoculation the streptococci had been subjected to temperatures of 50°, 56°, 60°, 65°, 70°, and 80° C for one hour in a water bath. Behrens (1923) concluded that there was a certain dilution of the bacterial suspension where the optimum resistance to heat was secured. A concentration greater or less than this resulted in a higher percentage of killing of the cells. Behrens worked with *Escherichia coli* and *Bacillus pyocyaneus* Gessard (1882). Fleischer and Amster (1923) have shown that the nature of the medium influences the thermal death points of the bacteria.

Harding (1923) reported daily fluctuations in bacterial counts during a day's run at large pasteurizing plants that indicate the presence of thermoduric bacteria. Ayers and Johnson (1923) pointed out that it would be unwise to attempt to transport milk in a hot condition because of the development of a thermophilic flora, some of which grow rapidly. Yates (1923), without considering the possibility of thermophilic bacteria, thought that the reaction of the media or the use of chlorine solutions was partly responsible for the presence of pin-point colonies on the agar plates. Robertson (1923), in a preliminary report on some non-spore-forming heat-resistant bacteria in pasteurized milk, isolated relatively large numbers of spherical bacteria, a few rods, and some species resembling *Streptococcus lactis* Löhnis. Yates and Glover (1923) found that the count of pasteurized milk at the end of a long run was greater than that of the raw milk as it left the receiving vat. Van Horn (1924) obtained about three times as high counts with the Difco beef extract agar as with the pre-war Leibig's beef extract agar when plating pasteurized milk. When plating raw milk the counts were only slightly higher on the Difco product.

Taylor, A. R. (1924) found that the milk leaving the holder was occasionally from 5.6 to 10.8 times the count of the same raw milk as it entered the preheater. Taylor, G. B. (1924) recognized two types of bacteria, one which was merely resistant to pasteurizing temperatures and one which grew rapidly at these temperatures. Hunger-

ford and Harding (1924) held samples of milk at the pasteurizing temperatures and found increased bacterial counts at the end of six hours. Tanner (1924a), using an enrichment method to make the thermophiles more abundant, isolated a number from milk. Adams and Harding (1924) secured thermophiles from 28.2 per cent of samples of common raw milk, 43.7 per cent of the Class "A" raw milk, and 40.4 per cent of the certified milk. Harding and Ward (1924) obtained samples from the storage vats of large city pasteurizing plants and found that by incubation at pasteurizing temperatures up to four and six hours a thermophilic flora could be developed.

Cooledge (1924) suggested the use of a medium with a pH of 7.2 for the growth of the non-spore-forming thermophilic flora. Morrison and Tanner (1922) isolated two cultures from a bottle of raw milk of excellent quality. Ayers and Johnson (1924a) pointed out the significance of the terms "majority" and "absolute" thermal death points of bacteria. Ayers and Johnson (1924b) described the organism causing the "pin-point" colonies and warned against the too broad use of the term "pin-point." The pH of the media and the crowded plates often give a plate characteristic of the "pin-point" colony condition. The causal organism was named *Lactobacillus thermophilus*. Fay (1927) has warned against the unrestricted use of the term "pin-point" colony as it superficially characterized the colony growth of several species of bacteria on the agar plates. Many of the common mesophiles, and some of the thermoduric flora and some of the true thermophilic species may form "pin-point" colonies. The writer has observed spore-forming thermophiles whose colonies also might be considered "pin-point" in size.

Johnson and Exworthy (1925) observed a streptococcus associated with abnormally high plate counts of pasteurized milk supplies. The organism could not be traced to improper pasteurization nor to unsanitary dairies. Swenarton (1925, 1926) associated the presence of "pin-point" colonies with milk produced in the spring. Fifty of the 52 cultures isolated were streptococci. No data are presented regarding their heat resistance. Tanner and H. G. Harding (1926a, 1926b) demonstrated thermophilic bacteria in all samples of milk after the milk had left the barn. The 73 cultures were Gram-positive, motile, spore-forming rods which grew well at pasteurizing temperatures. Several were strict thermophiles growing only above 37° C. More recently, Clarke and Dougherty (1926) have confirmed some of the observations on spore-forming and non-spore-forming ther-

mophilic species. Fay (1926, 1927) has described three types of thermoduric saccharophilic organisms which form characteristic "pin-point" colonies. These are probably members of the lactic acid group. Jenkins (1926) has found that certain common non-spore-forming species survive pasteurization temperatures also.

H. G. Harding (1927) has discussed the practical application of the facts which have recently been discovered regarding the occurrence and significance of thermoduric and thermophilic bacteria in milk.

SOURCE OF MATERIAL

Frequent reports of the presence of "pin-point" colonies of bacteria on milk control, agar plates were made at the Cleveland Meeting of the American Public Health Association (1923). This led the Committee on Standard Methods of the Laboratory Section to ask the Referee on Bacteriological Methods of Milk Analysis to make a study of the occurrence of these bacteria. Plates from Kansas City (Mo.), Detroit, Chicago, Milwaukee, Jackson (Mich.), Boston, and Baltimore were sent to the New York State Agricultural Experiment Station at Geneva. A total of 465 cultures were obtained from representative colonies on the pasteurized milk plates. In some cases where the probable growth of the colony was doubtful, a portion of the agar on which the colony was growing was put into the sterile milk tube.

Since only cultures that were resistant to pasteurizing temperatures were desired for further study, milk cultures grown at 25° C less than 24 hours old were exposed in Sternberg bulbs to a pasteurizing temperature. In this work, unless otherwise stated, a pasteurizing temperature of 145° F (62.8° C) for 30 minutes was used. The bulbs were immediately cooled to 40° F or less for 20 minutes and then the milk was plated with nutrient agar containing 1 per cent d-glucose and 0.1 per cent of a 0.001 per cent alcoholic solution of brom cresol purple. Only such plates as had a large number of colonies after incubation at 25° C for five days were recorded as resistant to pasteurizing temperatures. A summary of these results appear in Table 1.

In addition to the cultures secured in this way, Dr. D. H. Bergey of the University of Pennsylvania sent ten cultures of heat-resistant micrococci which he had isolated from milk. In the table, these are indicated by their source, Philadelphia.

By means of enrichment methods, the author isolated 132 cultures from the market milk supply of Geneva, N. Y., in 1925 and 41 from

the supply of Burlington, Vt., in 1927. These heat-resistant cultures were obtained by incubating samples from individual producers at

TABLE 1.—SOURCES OF THE 465 CULTURES FROM PASTEURIZED MILK PLATES.

DATE OF RECEIVING PLATES	PLATES SENT FROM	PLATE NO.	NUMBER OF ISOLATIONS FROM PLATE	NUMBER OF CULTURES SURVIVING PASTEURIZATION
Nov. 6, '22	Kansas City	1	15	15
Nov. 16, '22	Jackson, Mich.	2	7	4
Nov. 6, '22	Kansas City	3	14	10
Nov. 6, '22	Kansas City	4	14	2
Dec. 26, '22	Kansas City	5	6	1
Jan. 15, '23	Philadelphia	*	10	7
Dec. 26, '22	Kansas City	6	10	4
Dec. 26, '22	Kansas City	7	9	0
Nov. 28, '22	Kansas City	8	18	0
Jan. 26, '23	Chicago	9	9	2
Jan. 26, '23	Chicago	10	12	2
Jan. 26, '23	Chicago	11	13	6
Feb. 1, '23	Detroit	12	11	2
Feb. 1, '23	Detroit	13	12	2
Jan. 19, '23	Detroit	14	3	1
Jan. 19, '23	Detroit	15	3	0
Feb. 1, '23	Detroit	16	9	1
Feb. 1, '23	Detroit	17	8	1
Feb. 1, '23	Detroit	18	9	4
Feb. 1, '23	Detroit	19	7	1
Dec. 26, '22	Kansas City	20	6	1
Feb. 1, '23	Detroit	21	12	0
Feb. 16, '23	Boston	22	9	9
Feb. 16, '23	Boston	23	55	0
Feb. 16, '23	Boston	24	3	1
Jan. 23, '23	Milwaukee	25	13	6
Feb. 14, '23	Kansas City	26	12	9
Feb. 14, '23	Kansas City	27	18	14
Feb. 14, '23	Kansas City	28	18	13
Feb. 14, '23	Kansas City	29	7	3
Feb. 14, '23	Kansas City	30	6	6
Dec. 26, '22	Kansas City	31	4	2
Feb. 14, '23	Kansas City	32	10	2
Feb. 21, '23	Detroit	33	2	1
March 2, '23	Kansas City	34	6	0
March 2, '23	Kansas City	35	5	4
March 2, '23	Kansas City	36	6	4
March 2, '23	Kansas City	37	5	4
March 20, '23	Baltimore	38	8	1
March 20, '23	Baltimore	39	5	3
March 20, '23	Baltimore	40	4	3
March 20, '23	Baltimore	41	6	0
March 20, '23	Baltimore	42	12	11
March 16, '23	Chicago	43	5	5
March 16, '23	Chicago	44	4	4
March 16, '23	Chicago	45	4	4
March 16, '23	Chicago	46	5	5
March 16, '23	Chicago	47	7	7
March 16, '23	Chicago	48	9	9

*Isolated cultures were sent from Philadelphia.

145° F for a period of seven hours. Plates were prepared hourly and the counts obtained as shown in Table 2. Four of the 30 Geneva samples and 1 of the 30 Burlington samples showed an increase in the count during the holding period. The colonies of the types which increased in numbers were small, round, and dull white. They might be described as "pin-point" colonies. An examination of the cultures made from representative colonies revealed that of the 132 Geneva cultures and of the 41 Burlington cultures, 97 and 34, respectively, were rods representing the same species. The remaining cultures were either yellow or white cocci with the yellow ones predominating. The data on which these conclusions are based will be discussed later (page 28).

TABLE 2.—CHANGES IN THE PLATE COUNT OF SAMPLES OF MILK HELD AT PASTEURIZING TEMPERATURES.

SAMPLE No.	TIME OF HOLDING							
	0 hours	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours
	Geneva, N. Y., Milk Supply*							
1	113,000	3,000	7,000	23,000	47,000	18,000	15,000	26,000
2	540,000	21,600	16,000	40,000	114,000	52,000	67,000	28,000
3	24,000	6,000	17,000	81,000	103,000	74,000	58,000	67,000
4	140,000	20,400	26,600	69,000	218,000	141,000	121,000	93,000
	Burlington, Vt., Milk Supply†							
1	193,000	51,000	17,600	44,000	151,000	167,000	123,000	88,000

*26 other samples tested but no growth attributable to non-spore-forming thermophiles appeared.
†29 other samples tested but no growth attributable to non-spore-forming thermophiles appeared.

By accident on two different occasions in an attempt to obtain thermophiles by incubating samples of milk at 145° F for three days, a culture of a yellow sarcina developed after the samples had stood at room temperature for an additional five days. Because of their peculiar heat resistance, these two cultures, Nos. 1Z and 2Z, have been included in this discussion among the cultures spoken of as Type III (page 49).

During the course of this work, samples of milk from various sources were frequently incubated at pasteurizing temperatures for several days at a time. Usually, spore-forming thermophiles developed. The difficulties of isolating non-spore-forming thermophiles are far greater than those encountered in the isolation of the spore-forming type.

H. G. Harding sent some pasteurized milk plates from Madison, Wis., to the Geneva laboratory in December, 1925. The material

was broken in transit so that no isolations could be made directly. Parts of the agar were placed in three sterile milk tubes and incubated at 25° C for a few hours. The milk tubes were then placed at pasteurizing temperatures for a period of five hours. Plates were prepared at the end of the third, fourth, and fifth hours. Practically a pure culture of a pink sarcina developed on all of the plates from each of the three inoculated tubes. Type V (page 50) consisted of 21 isolations made from these plates. Cultures H1 and H19 were retained for further study.

A total of 415 out of 721 milking machine cultures isolated in a previous investigation, Robertson (1924), were available and milk cultures of these were pasteurized. These results will be discussed in their proper sequence (pages 29 to 39).

The natural habitat of the cultures under observation cannot be determined accurately. Probably they represent either water, air, or soil species. Frequently they were found in practically pure cultures on the pasteurized milk plates. The isolations of the heat-resistant species from the Geneva milk supply suggest that they may be found in the milk of one out of every 7 or 8 producers, while those from Burlington indicate that they may be isolated from the milk of one out of every 30 producers.

TECHNIC

The following technic has been used thruout this work. Observations were made as to the grouping of the cells both from the 24-hour-old milk cultures and from the growth on agar slants. The preparations from the agar slant growth were stained by the Gram method, while the milk preparations were made and stained according to the Breed method. Morphology was observed on successive days in some unusual cultures. The Gram stain was made with the Hucker (1921) ammonium oxalate method. Chromogenesis was determined on the two-weeks' old agar slant. Motility was studied in a culture less than 24 hours old.

The ability of the cultures to use carbohydrates and organic acids was determined by adding 1 per cent of the desired substance to either nutrient agar or nutrient broth. The medium was then neutralized and 0.1 per cent each of brom cresol purple (0.001 per cent alcoholic) and cresol red (0.001 per cent alcoholic) were added.

The ability to use purine bases, ammonia nitrogen, amino acid nitrogen, and urea was determined in neutralized media of the following composition with indicators added as before:

Distilled water	1,000 cc
Potassium chloride	2 grams
Magnesium sulfate	1 gram
D-glucose	10 grams
Nitrogen-containing substance	10 grams

Growth and acid production were observed on each of the first five days and again on the tenth day. In some of the work nutrient broth was substituted for nutrient agar. The reduction of nitrates to nitrites was determined on nutrient agar to which 0.1 per cent of potassium nitrate had been added. Determinations of the presence of nitrites were made by adding a few drops of sulfanilic acid and α-naphthylamine solutions to the agar slants on the first, second, and fifth days after inoculation. Growth and the amount of gelatin liquefaction at 18° C were observed for a period of three months. Liquefaction within 5 days was recorded as rapid, 5 to 15 days as moderate, and over 15 days as slow. Failure to liquefy at the end of three months was regarded as indicating a non-liquefying culture. The gelatin tubes were sealed with paraffin to prevent drying out.

The growth in litmus milk was observed daily for ten days and with some unusual cultures which failed to alter the milk in ten days the period was extended to 20 days. The rapidity with which the litmus was reduced was assumed to be an indication of the rate of growth in those cultures which had the faculty of reducing litmus. Coagulation and peptonization was recorded as soon as noticed. The relation to oxygen requirements was observed as far as possible from the location of growth in Smith fermentation tubes.

SELECTION OF CULTURES

The characters of the 197 cultures which survived the pasteurization treatment appear in Table 3. These data record the morphology, chromogenesis, Gram stain reactions, relation to oxygen, the power to liquefy gelatin and to reduce nitrates, the production of acid on d-glucose, lactose, and sucrose, the ability to use ammonia and amino acid nitrogen, and the reaction in litmus milk.

Table 4 gives the distribution of these cultures according to their morphology, chromogenesis, action on gelatin, and reduction of nitrates. The four largest groups were (1) the non-liquefying, non-nitrate-reducing white rods; (2) the liquefying, nitrate-reducing yellow cocci; (3) the liquefying, non-nitrate-reducing yellow cocci; and (4) the rods resembling *Streptococcus lactis*. Of these 197 cultures there

TABLE 3.—CHARACTERS OF THE 197 CULTURES FROM THE PASTEURIZED MILK PLATES WHICH SURVIVED PASTEURIZATION IN STERNBERG BULBS.

Culture No.	History			Cultural characters												
										Fermentation of			Milk reactions			
	Date of receiving plates	Source*	Plate No.	Morphology†	Chromogenesis‡	Gram stain	Aerobe	Gelatin liquefaction	Nitrate reduction	D-glucose	Lactose	Sucrose	Acid production	Acid production and coagulation	Peptonization	Utilization of ammonia nitrogen
1	Nov. 6, '22	K	1	C	y	+	+	+	+	+	+	—	+	—	—	+
2	Nov. 6, '22	K	1	C	y	+	+	+	+	+	+	+	+	—	—	+
3	Nov. 6, '22	K	1	C	y	+	+	+	+	+	+	—	+	—	—	+
4	Nov. 6, '22	K	1	R	w	+	+	—	—	+	+	+	+	—	—	+
5	Nov. 6, '22	K	1	C	y	+	+	+	+	+	+	+	+	—	—	+
6	Nov. 6, '22	K	1	C	y	+	+	+	+	+	+	+	+	—	—	?
7	Nov. 6, '22	K	1	C	y	+	+	+	+	+	+	+	+	—	—	?
8	Nov. 6, '22	K	1	C	y	+	+	+	+	+	+	+	+	—	—	?
9	Nov. 6, '22	K	1	R	w	+	+	—	—	+	+	+	+	—	—	?
9C	Nov. 6, '22	K	1	C	y	+	+	+	+	+	+	+	+	—	—	+
10	Nov. 6, '22	K	1	R	w	+	+	—	—	+	+	+	+	—	—	?
11	Nov. 6, '22	K	1	R	w	+	+	—	—	+	+	+	+	—	—	?
12	Nov. 6, '22	K	1	C	y	+	+	+	+	+	+	+	+	—	—	?
13	Nov. 6, '22	K	1	C	y	+	+	+	+	+	+	+	+	—	—	?
14C	Nov. 6, '22	K	1	C	y	+	+	+	+	+	+	+	+	—	—	+
15	Nov. 16, '22	J	2	R	w	—	+	—	+	—	—	—	—	—	+	?
16	Nov. 16, '22	J	2	R	w	—	+	—	+	—	—	—	—	—	+	?
17	Nov. 16, '22	J	2	R	w	+	+	—	—	+	+	+	+	—	—	?
18	Nov. 16, '22	J	2	C	w	+	+	—	—	+	+	+	+	+	—	—
19	Nov. 6, '22	K	3	C	y	+	+	+	—	—	—	—	+	—	—	+
20R	Nov. 6, '22	K	3	R	w	+	+	—	—	+	+	+	+	—	—	+
20Y	Nov. 6, '22	K	3	C	y	+	+	+	—	—	—	—	+	—	—	+
21	Nov. 6, '22	K	3	C	y	+	+	+	—	—	—	—	+	+	—	+
22	Nov. 6, '22	K	3	C	y	+	+	—	+	+	+	—	+	—	—	+
23	Nov. 6, '22	K	3	C	y	+	+	+	—	—	—	—	+	—	—	?
23R	Nov. 6, '22	K	3	C	w	+	+	—	—	+	+	+	+	+	—	—

24	Nov. 6, '22	K	3	C	w	+	+	—	—	+	+	+	+	+	—	?
25	Nov. 6, '22	K	3	C	w	+	+	—	—	+	++	++	++	++	—	?
26	Nov. 6, '22	K	4	C	w	+	+	—	—	+	++	+	+	+	—	?
27	Nov. 6, '22	K	4	C	y	+	+	+	—	—	—	—	+	—	—	?
28	Dec. 26, '22	K	5	C	y	+	+	—	+	+	+	+	+	—	—	?
29	Jan. 15, '23	P	—	C	w	+	+	—	—	+	—	+	+	—	—	?
30	Jan. 15, '23	P	—	C	y	+	+	—	—	+	—	+	—	—	+	?
31	Jan. 15, '23	P	—	C	w	+	+	+	—	+	—	+	—	—	+	?
32	Jan. 15, '23	P	—	C	w	+	+	+	—	+	—	+	—	—	+	?
33	Jan. 15, '23	P	—	C	y	+	+	+	—	+	—	+	—	—	+	?
34	Jan. 15, '23	P	—	C	y	+	+	—	—	+	—	+	—	—	+	?
35	Nov. 6, '22	K	3	C	w	+	+	—	—	+	+	+	+	+	—	—
36	Jan. 15, '23	P	—	C	y	+	+	—	—	+	—	+	—	—	+	+
37	Dec. 26, '22	K	6	C	y	+	+	+	+	+	+	+	+	—	—	+
38	Dec. 26, '22	K	6	C	y	+	+	+	+	+	+	+	+	—	—	?
39	Dec. 26, '22	K	6	C	y	+	+	—	+	+	+	+	+	—	—	?
40	Dec. 26, '22	K	6	C	w	+	+	—	—	+	+	+	+	+	—	?
41	Jan. 26, '23	C	9	C	w	+	+	—	—	+	+	+	+	+	—	?
42	Jan. 26, '23	C	9	C	w	+	+	—	—	+	+	+	+	+	—	?
43	Jan. 26, '23	C	10	C	w	+	+	—	—	+	+	+	+	+	—	?
44	Jan. 26, '23	C	10	C	w	+	+	—	—	+	+	+	+	+	—	?
45	Jan. 26, '23	C	11	C	w	+	+	—	—	+	+	+	+	+	—	?
46	Jan. 26, '23	C	11	C	w	+	+	—	—	+	+	+	+	+	—	?
47	Jan. 26, '23	C	11	C	w	+	+	—	—	+	+	+	+	+	—	?
48	Jan. 26, '23	C	11	C	w	+	+	—	—	+	+	+	+	+	—	?
49	Jan. 26, '23	C	11	C	w	+	+	—	—	+	+	+	+	—	—	?
50	Jan. 26, '23	C	11	C	w	+	+	—	—	+	+	+	+	+	—	?
51	Feb. 1, '23	D	12	C	w	+	+	—	—	+	+	+	+	—	—	?
52	Feb. 1, '23	D	12	C	w	+	+	—	—	+	+	+	+	+	—	?
53	Feb. 1, '23	D	13	C	w	+	+	—	—	+	+	+	+	+	—	?
54	Feb. 1, '23	D	13	C	w	+	+	—	—	+	+	+	+	+	—	?
55	Jan. 19, '23	D	14	C	w	+	+	—	—	+	+	+	+	+	—	?
56	Feb. 1, '23	D	16	C	w	+	+	—	—	+	+	+	+	+	—	?
57	Feb. 1, '23	D	17	C	w	+	+	+	+	+	+	+	+	+	—	?
58	Feb. 1, '23	D	18	C	w	+	+	—	—	+	+	+	+	—	—	?
59	Feb. 1, '23	D	18	C	w	+	+	—	—	+	+	+	+	+	—	?

*Letters indicate as follows:
K—Kansas City, Mo.
J—Jackson, Mich.
P—Philadelphia, Pa.
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D—Detroit, Mich.
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M—Milwaukee, Wis.
Ba—Baltimore, Md.

†C—coccus form; R—rod form.
‡w—white; y—yellow.

TABLE 3.—*Continued.*

Culture No.	History			Cultural characters												
										Fermentation of			Milk reactions			
	Date of receiving plates	Source*	Plate No.	Morphology†	Chromogenesis‡	Gram stain	Aerobe	Gelatin liquefaction	Nitrate reduction	D-glucose	Lactose	Sucrose	Acid production	Acid production and coagulation	Peptonization	Utilization of ammonia nitrogen
60	Feb. 1, '23	D	18	C	w	+	+	—	—	+	+	+	+	—	—	?
61	Feb. 1, '23	D	18	C	w	+	+	—	—	+	+	+	+	—	—	?
62	Feb. 1, '23	D	19	C	w	+	+	—	—	+	+	+	+	—	—	?
63	Dec. 26, '22	K	20	C	w	+	+	—	—	+	+	+	+	—	—	?
64	Feb. 16, '23	B	22	C	w	+	+	—	—	+	+	+	+	+	—	?
65	Feb. 16, '23	B	22	C	y	+	+	+	+	+	+	+	+	—	—	—
66	Feb. 16, '23	B	22	C	y	+	+	+	+	+	+	+	+	—	—	?
67	Feb. 16, '23	B	22	R	w	—	+	—	—	—	—	—	+	—	—	?
68	Feb. 16, '23	B	22	C	y	+	+	+	+	+	+	+	+	—	—	—
69	Feb. 16, '23	B	22	C	w	+	+	+	—	+	+	+	+	+	—	?
70	Feb. 16, '23	B	22	C	y	+	+	+	+	+	+	+	+	—	—	+
71	Feb. 16, '23	B	22	R	w	—	+	—	+	+	—	—	—	—	+	?
72	Feb. 16, '23	B	22	C	w	+	+	—	—	+	+	+	+	+	—	—
73	Feb. 16, '23	B	24	C	w	+	+	—	+	+	+	—	+	—	—	?
74	Jan. 23, '23	M	25	C	w	+	+	—	—	+	+	+	+	+	—	?
75	Jan. 23, '23	M	25	R	w	+	+	—	—	+	+	+	+	—	—	+
76	Jan. 23, '23	M	25	R	w	+	+	+	—	+	+	+	+	—	—	?
77	Jan. 23, '23	M	25	C	w	+	+	+	+	+	+	+	+	+	—	?
78	Jan. 23, '23	M	25	C	w	+	+	+	+	+	+	+	+	+	—	?
79	Jan. 23, '23	M	25	R	y	+	+	+	—	+	+	+	+	+	—	?
80	Feb. 14, '23	K	26	C	y	+	+	—	+	+	+	+	+	—	—	?
81	Feb. 14, '23	K	26	C	y	+	+	—	+	+	+	+	+	+	—	?
82	Feb. 14, '23	K	26	R	w	+	+	—	—	+	+	+	+	—	—	?
83	Feb. 14, '23	K	26	C	w	+	+	—	—	+	+	+	+	+	—	?
84	Feb. 14, '23	K	26	C	y	+	+	+	+	—	—	—	+	—	—	?
85	Feb. 14, '23	K	26	R	w	+	+	—	—	+	+	+	+	—	—	—
86	Feb. 14, '23	K	26	C	y	+	+	+	—	—	—	—	+	—	—	+

87	Feb. 14, '23	K	26	R	w	+	+	−	−	+	+	+	+	−	−	−	+
88	Feb. 14, '23	K	26	C	y	+	+	−	−	+	+	+	+	−	−	−	+
89	Feb. 14, '23	K	27	R	w	+	+	+	−	−	−	+	+	−	−	−	?
89A	Feb. 14, '23	K	27	C	y	+	+	−	−	+	+	+	+	−	−	−	+
90	Feb. 14, '23	K	27	R	y	+	+	+	−	−	−	−	+	−	−	−	?
90A	Feb. 14, '23	K	27	C	y	+	+	+	+	+	+	+	+	−	−	−	+
91	Feb. 14, '23	K	27	C	y	+	+	+	−	+	+	+	+	−	−	−	+
92	Feb. 14, '23	K	27	C	w	+	+	+	+	+	+	−	+	−	−	−	+
93	Feb. 14, '23	K	27	C	w	+	+	−	−	+	+	+	+	−	−	−	+
93A	Feb. 14, '23	K	27	R	y	+	+	−	−	+	+	+	+	−	−	−	?
94	Feb. 14, '23	K	27	R	w	+	+	+	−	−	−	−	+	−	−	−	?
94A	Feb. 14, '23	K	27	C	y	+	+	−	−	+	+	+	+	−	−	−	+
95	Feb. 14, '23	K	27	R	w	+	+	+	+	+	+	−	+	−	−	−	+
95A	Feb. 14, '23	K	27	C	y	+	+	−	−	+	+	+	+	−	−	−	+
96	Feb. 14, '23	K	27	R	w	+	+	+	+	+	+	+	+	−	−	−	+
96+	Feb. 14, '23	K	27	C	y	+	+	−	−	+	+	+	+	−	−	−	+
97	Feb. 14, '23	K	28	R	w	+	+	−	+	+	+	−	+	−	−	−	?
97A	Feb. 14, '23	K	28	C	w	+	+	−	−	+	+	+	+	−	−	−	+
98	Feb. 14, '23	K	28	R	y	+	+	−	−	+	+	+	+	−	−	−	+
98A	Feb. 14, '23	K	28	R	y	+	+	−	+	+	+	−	+	−	−	−	+
98B	Feb. 14, '23	K	28	C	w	+	+	+	+	+	+	+	+	−	−	−	+
98C	Feb. 14, '23	K	28	C	y	+	+	−	−	+	+	+	+	−	−	−	+
99	Feb. 14, '23	K	28	R	w	+	+	+	−	+	−	+	+	−	−	−	+
99A	Feb. 14, '23	K	28	C	y	+	+	−	−	+	+	+	+	−	−	−	+
100	Feb. 14, '23	K	28	R	y	+	+	+	−	+	+	+	+	−	−	−	?
100A	Feb. 14, '23	K	28	C	y	+	+	+	+	+	+	−	+	−	−	−	?
101	Feb. 14, '23	K	28	C	y	+	+	+	+	+	+	−	+	−	−	−	?
102	Feb. 14, '23	K	28	C	w	+	+	−	−	+	+	+	+	+	−	−	?
103	Feb. 14, '23	K	28	C	w	+	+	−	−	+	+	+	+	−	−	−	−
104	Feb. 14, '23	K	29	R	y	+	+	−	−	+	+	+	+	−	−	−	?
105	Feb. 14, '23	K	29	R	y	+	+	+	+	+	+	+	+	+	−	−	?
106	Feb. 14, '23	K	29	C	w	+	+	+	+	+	+	+	+	−	−	−	?
107C	Feb. 14, '23	K	30	C	y	+	+	−	−	+	+	+	+	−	−	−	+
107R	Feb. 14, '23	K	30	R	y	+	+	+	+	+	+	+	+	−	−	−	?
108	Feb. 14, '23	K	30	C	y	+	+	+	+	+	+	+	+	−	−	−	?
109	Feb. 14, '23	K	30	C	y	+	+	+	+	+	+	+	+	−	−	−	+
110	Feb. 14, '23	K	30	C	y	+	+	+	+	+	+	+	+	+	−	−	+
111	Feb. 14, '23	K	30	C	w	+	+	+	+	+	+	+	+	+	−	−	?
112	Feb. 14, '23	K	31	C	y	+	+	−	−	+	+	+	+	−	−	−	?
113	Feb. 14, '23	K	31	R	w	+	+	+	+	+	+	+	+	−	−	−	+

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M—Milwaukee, Wis.
Ba—Baltimore, Md.
†C—coccus form; R—rod form.
‡w—white; y—yellow.

TABLE 3.—*Concluded.*

Culture No.	History			Cultural characters												
	Date of receiving plates	Source*	Plate No.	Morphology†	Chromogenesis‡	Gram stain	Aerobe	Gelatin liquefaction	Nitrate reduction	Fermentation of			Milk reactions			Utilization of ammonia nitrogen
										D-glucose	Lactose	Sucrose	Acid production	Acid production and coagulation	Peptonization	
114	Feb. 21, '23	D	33	C	y	+	+	+	+	+	+	+	+	—	—	?
115	Dec. 26, '22	K	32	C	w	+	+	—	—	+	+	+	+	—	—	?
116	Dec. 26, '22	K	32	C	w	+	+	—	—	+	+	+	+	—	—	—
117	March 2, '23	K	35	C	w	+	+	—	—	+	+	+	+	+	—	?
118	March 2, '23	K	35	C	w	+	+	—	—	+	+	+	+	+	—	?
119	March 2, '23	K	35	C	w	+	+	—	—	+	+	+	+	+	—	?
120	March 2, '23	K	35	C	w	+	+	—	—	+	+	+	+	+	—	?
121	March 2, '23	K	36	C	w	+	+	—	—	+	+	+	+	+	—	?
122	March 2, '23	K	36	C	w	+	+	—	—	+	+	+	+	+	—	?
123	March 2, '23	K	36	C	w	+	+	—	—	+	+	+	+	+	—	—
124	March 2, '23	K	36	C	w	+	+	—	—	+	+	+	+	+	—	?
125	March 2, '23	K	37	C	w	+	+	—	—	+	+	+	+	+	—	?
126	March 2, '23	K	37	C	w	+	+	—	—	+	+	+	+	+	—	?
127	March 2, '23	K	37	C	w	+	+	—	—	+	+	+	+	+	—	?
128	March 2, '23	K	37	C	w	+	+	—	—	+	+	+	+	+	—	?
129	March 20, '23	Ba	38	C	w	+	+	—	—	+	+	+	+	—	—	?
130	March 20, '23	Ba	39	R	w	+	+	—	—	+	+	+	+	—	—	?
131	March 20, '23	Ba	39	C	y	+	+	+	+	+	+	+	+	—	—	?
132	March 20, '23	Ba	39	C	y	+	+	+	+	+	+	+	+	—	—	?
133	March 20, '23	Ba	40	C	w	+	+	+	+	+	+	+	+	+	—	?
134	March 20, '23	Ba	40	C	w	+	+	—	—	+	+	+	+	—	—	?
135	March 20, '23	Ba	40	C	w	+	+	—	—	+	+	+	+	—	—	?
136	March 20, '23	Ba	42	R	w	+	+	—	—	+	+	+	+	—	—	?
137C	March 20, '23	Ba	42	C	y	+	+	+	+	+	+	+	+	—	—	+
138	March 20, '23	Ba	42	C	y	+	+	+	—	+	+	+	+	—	—	?
139	March 20, '23	Ba	42	R	w	+	+	—	—	+	+	+	+	—	—	?
140	March 20, '23	Ba	42	C	y	+	+	+	—	+	+	+	+	—	—	?

141Y	March 20, '23	Ba	42	C	y	+	+	+	+	+	+	+	+	−	−	+
141R	March 20, '23	Ba	42	R	w	+	+	−	−	+	+	+	+	−	−	+
142	March 20, '23	Ba	42	C	y	+	+	−	−	+	+	−	+	−	−	?
143	March 20, '23	Ba	42	C	w	+	+	−	−	+	−	−	+	−	−	?
144	March 20, '23	Ba	42	R	w	+	+	−	−	+	+	+	+	−	−	?
145	March 20, '23	Ba	42	C	y	+	+	−	+	+	+	−	+	−	−	?
146	March 16, '23	C	43	R	w	+	+	−	−	+	+	+	+	−	−	?
147	March 16, '23	C	43	C	w	+	+	−	−	+	+	−	+	−	−	?
148	March 16, '23	C	43	C	w	+	+	+	−	+	+	+	+	+	−	?
149	March 16, '23	C	43	C	w	+	+	+	−	+	+	+	+	+	−	?
150	March 16, '23	C	43	C	w	+	+	+	−	+	+	+	+	+	−	?
151	March 16, '23	C	44	C	w	+	+	+	−	+	+	+	+	+	−	?
152	March 16, '23	C	44	C	w	+	+	−	−	+	+	+	+	+	−	?
153	March 16, '23	C	44	C	w	+	+	−	−	+	+	+	+	−	−	?
154	March 16, '23	C	44	C	w	+	+	−	−	+	+	+	+	+	−	?
155	March 16, '23	C	45	C	w	+	+	−	−	+	+	+	+	−	−	?
156	March 16, '23	C	45	C	w	+	+	−	−	+	+	+	+	−	−	?
157	March 16, '23	C	45	C	w	+	+	−	−	+	+	+	+	−	−	?
158	March 16, '23	C	45	C	w	+	+	−	−	+	+	+	+	−	−	?
159	March 16, '23	C	46	C	w	+	+	−	−	+	+	+	+	+	−	?
160	March 16, '23	C	46	C	w	+	+	−	−	+	+	+	+	−	−	?
161	March 16, '23	C	46	C	w	+	+	−	−	+	+	+	+	−	−	?
162	March 16, '23	C	46	C	w	+	+	−	−	+	+	+	+	−	−	?
163	March 16, '23	C	46	C	w	+	+	−	−	+	+	+	+	−	−	?
165	March 16, '23	C	47	C	w	+	+	−	−	+	+	+	+	−	−	?
166	March 16, '23	C	47	C	w	+	+	−	−	+	+	+	+	−	−	?
167	March 16, '23	C	47	C	w	+	+	−	−	+	+	+	+	−	−	?
168	March 16, '23	C	47	C	w	+	+	−	−	+	+	+	+	−	−	?
169	March 16, '23	C	47	C	w	+	+	−	−	+	+	+	+	+	−	?
170	March 16, '23	C	47	C	w	+	+	−	−	+	+	+	+	−	−	?
171	March 16, '23	C	47	C	w	+	+	−	−	+	+	+	+	−	−	?
172	March 16, '23	C	48	C	w	+	+	−	−	+	+	+	+	−	−	?
173	March 16, '23	C	48	C	w	+	+	−	−	+	+	+	+	−	−	?
174	March 16, '23	C	48	C	w	+	+	−	−	+	+	+	+	−	−	?
175	March 16, '23	C	48	C	w	+	+	+	−	+	+	+	+	−	−	?
176	March 16, '23	C	48	C	w	+	+	−	−	+	+	+	+	−	−	?
177	March 16, '23	C	48	C	w	+	+	−	−	+	+	+	+	−	−	?
178	March 16, '23	C	48	C	w	+	+	−	−	+	+	+	+	−	−	?
179	March 16, '23	C	48	C	w	+	+	−	−	+	+	+	+	−	−	?
180	March 16, '23	C	48	C	w	+	+	−	−	+	+	+	+	−	−	?

*Letters indicate as follows:
K—Kansas City, Mo.
J—Jackson, Mich.
P—Philadelphia, Pa.
C—Chicago, Ill.
D—Detroit, Mich.
B—Boston, Mass.
M—Milwaukee, Wis.
Ba—Baltimore, Md.
†C—coccus form; R—rod form.
‡w—white; y—yellow.

TABLE 4.—DISTRIBUTION OF THE 197 HEAT-RESISTANT CULTURES FROM PASTEURIZED MILK PLATES GROUPED ACCORDING TO MORPHOLOGY, CHROMOGENESIS, GELATIN LIQUEFACTION, AND NITRATE REDUCTION.

MORPHOLOGY AND CHROMOGENESIS	GELATIN LIQUEFACTION				TOTAL NUMBER OF CULTURES
	Positive		Negative		
	Nitrate reduction positive	Nitrate reduction negative	Nitrate reduction positive	Nitrate reduction negative	
Coccus, yellow.....	38	15	8	5	66
Coccus, white.....	4	8	1	2	15
Rods, yellow.....	0	1	0	0	1
Rod, white.....	0	1	3	32	36
Types resembling *Streptococcus lactis*.	0	0	0	79	79

were 37 rods, 79 resembling *Streptococcus lactis*, and 81 cocci. Only 15 of these cocci were white, the remainder being yellow. Only one of the rods was yellow, the remainder being white. The white rods were difficult to grow on the agar slants, a fact which explains the failure to obtain more cultures of this type. Cultures of this type were relatively common on the pasteurized milk plates. A similar classification of Ayers and Johnson's (1913) cultures appears in Table 5.

THERMAL RESISTANCE OF SPECIES STUDIED

The selection of the above heat-resistant cultures depended merely on the ability of an unknown number of organisms in 1 cc of milk to survive pasteurization in Sternberg bulbs. Representative cultures, therefore, were again selected from the series and more accurate data obtained to show the degree of resistance.

The literature is burdened with data based solely on a few tests on a milk culture, usually in open tubes, telling that this and that species is heat resistant. Such data do not indicate whether 5 or 95 per cent of the individual cells survive a given temperature. Gage and Stoughton (1906) report that the killing range is frequently as much as 35°C. The futility of such data appears more forcibly in Table 9. Only data where the thermal death point determinations or quantitative determinations of the numbers surviving are accurately made are of real value, and even these should be qualified with the conditions under which the tests were made.

Because of the bearing which this work has on pasteurization of milk, pure milk cultures, 15 hours old, have been pasteurized with this thought in mind. A DeKhotinsky electrically controlled water bath was provided to insure greater accuracy. The cultures were

TABLE 5.—DISTRIBUTION OF AYERS AND JOHNSON'S CULTURES FROM PASTEURIZED MILK ACCORDING TO MORPHOLOGY, CHROMOGENESIS, GELATIN LIQUEFACTION, AND NITRATE REDUCTION.*

MORPHOLOGY	CHROMOGENESIS	GELATIN LIQUEFACTION						TOTAL NUMBER OF CULTURES
		Positive			Negative			
		Nitrate reduction			Nitrate reduction			
		Positive	Negative	Untested	Positive	Negative	Untested	
		Acid-forming Group						
Coccus	±	0	0	0	118	0	0	118
Coccus	—	0	0	9	0	0	0	9
Rod	—	0	1	0	0	0	0	1
Rod	—	0	0	0	0	0	4	4
		Alkali-forming Group						
Coccus	±	0	0	0	0	0	31	31
Rod	±	0	0	0	0	0	12	12
		Peptonizing Group						
Coccus	±	0	3	0	0	0	0	3
?	±	0	0	8	0	0	0	9
?	±	0	0	0	0	0	14	14
?	±	0	0	25	0	0	0	25
		Acid-forming Group						
Coccus	—	4	0	0	1	13	5	27
Coccus	+	7	9	5	0	0	0	21
Rod	—	1	0	0	0	0	0	1
Rod	yellow	0	0	0	0	3	0	3
		Alkali-forming Group						
Coccus	—	0	0	0	1	2	0	3
Coccus	yellow	0	0	0	0	1	0	1
Rod	—	0	0	0	1	1	0	2
Rod	yellow	0	0	0	1	0	0	1
		Peptonizing Group						
Coccus	white	3	2	0	1	2	0	8
Coccus	yellow	7	5	0	0	2	0	14
Coccus	brown	0	1	0	0	0	0	1
Rod	white	6	4	0	1†	1	0	12
Rod	yellow	1	0	0	0	1	0	2

*Adapted from *United States Dept. Agr. Bur. Animal Ind. Bul. No. 161.*
†Spore-forming rod.

TABLE 6.—PERCENTAGE INCREASE OR DECREASE IN PLATE COUNT AFTER HEATING AS INFLUENCED BY SUBJECTING MILK CULTURES OF SELECTED HEAT-RESISTANT BACTERIA TO 62.8°C FOR 30 MINUTES.

CULTURE No.	TRIAL NUMBER							
	1	2	3	4	5	6	7	8
1	—38.4	—86.0	—53.7	—41.7	—42.9	—35.7		
2	+23.9	—92.0	—36.5	—30.0	—25.0	—27.6		
3	—99.9	—99.9						
4	—77.7	—99.0						
5	—99.8	—80.0						
6	—99.3	—98.4	—98.6	—98.8	—98.9	—89.0		
7	—99.0	—73.0						
9	—27.7	—95.0						
9C	—32.7	—24.0	— 9.6	—22.7				
10	—99.9	—64.0	—99.9	—99.9	—99.9	—99.9		
11	+ 7.2	—92.0	—99.9	—99.9	—99.9	—99.9		
12	—99.6	—86.8	—88.9	—89.7	—83.5	—87.0		
13	—95.0							
14C	—20.0	—10.0	—24.0	—50.0				
15	—79.0	—86.0	—78.0	—80.0	—97.9	—50.0		
16	—99.9	—99.9	—99.9	—99.9	—99.9	—45.0		
17	+13.0	+ 6.5	—26.0	—23.4	—26.5	+10.1		
18	+23.6	+ 0.9	— 2.7	—15.0	—15.1			
19	+13.1	+16.4	+ 7.0	+ 5.0	—17.0	—19.2	—22.0	—27.1
20R	+18.4	+ 5.1	+ 3.2	— 1.4	— 3.9	— 9.5	— 7.4	—13.1
20Y	+11.4	+21.2	+13.1	+ 7.2	+ 5.1	— 7.0	—13.2	—18.1
21	—43.0	—60.0	—11.9	—26.7	—20.0	— 9.4	+ 8.5	+ 9.0
22	— 5.0	—42.0	—56.0	—54.0	—20.0	—55.6		
23	—44.0	—17.2	— 2.3	— 1.3	— 3.7			
23R	+38.7	+26.4	+23.6	— 9.3	—00.0	+ 8.5	— 2.3	— 1.3
27	—37.0	—96.5	—52.0	—53.1	—42.9	—53.4		
28	—89.3	—99.9	—99.9	—99.9	—99.9			
29	—23.0	—93.9	—92.4	—96.8	—86.9			
30	—39.0	—71.0	+53.0	—23.0	—15.0	—61.0	—37.8	—33.7
31	—61.0	—99.9	—99.9	—99.9	—99.9			
32	—20.0	+15.0	— 7.0					
33	—61.0	—99.9	—99.9	—99.9	—99.9			
34	+ 5.0	—22.0	—42.2	—32.1	+14.1	—56.6	—26.8	—11.2
35	+12.4	— 1.1	—40.0	— 0.9	—13.0	—27.8	—23.9	+ 4.7
36	—37.0	—17.0	—15.0	—25.0	—60.0			
37	—54.0	—99.9	—97.4	—96.7	—98.7			
38	—99.9	—99.9	—99.9	—99.9	—99.9			
39	—72.0	—98.0	—98.0	—98.0	—98.0			
56	—99.9	—99.9	—99.9	—99.9				
58	—99.9	—99.9	—99.9	—99.9				
66	—99.9	—99.9	—99.9	—99.9				
67	—99.9	—99.9	—99.9	—99.9				
68	—99.9	—99.9	—99.9	—99.9				
69	—99.9	—99.9	—99.9	—99.9				
70	+70.0	+25.9	+ 1.6	—14.1	—13.1	—27.4	—25.0	—28.1
72	+10.3	+13.4	+12.4	— 0.0	+ 6.8	+ 9.7	— 1.3	+34.1
75	+12.9	+15.9	—10.0	— 4.8	— 6.8	— 9.9	— 4.8	—21.6
76	—99.0							
77	—50.0	—52.0	—58.0	—60.0	—11.6	— 4.4	—17.8	— 6.8
78	—95.0	—95.0	—96.0	—95.0				
80	— 3.0	—16.8	— 6.3	—12.3				
85	— 3.0	— 0.3	—12.3	—16.0				
86	—99.9	—99.9	—99.9	—99.9				
87	+17.9	— 4.9	+ 0.0	— 5.0	— 4.1	—13.0	— 9.8	— 3.9
88	—46.5	—32.2	—39.9	—39.3				
89	—26.4	—21.1	+ 3.4	+14.0	+11.1	—24.7	—22.2	—36.4
90	—44.9	—49.8	—30.0	—32.9				

TABLE 6.—*Continued.*

CULTURE No.	TRIAL NUMBER							
	1	2	3	4	5	6	7	8
91	— 99.9	—99.9	—99.9	—99.9				
93	— 99.9	—99.9	—99.9	—99.9				
94	+ 2.1	— 3.0	—15.4	—12.5	—21.9	—22.3	—20.6	—15.8
95	— 99.9	—99.9	—99.9	—99.9	—47.0			
95A	— 99.9	—99.9	—99.9	—99.9				
96	— 95.0	—95.0	—95.0	—95.0				
96+	— 13.8	+ 0.5	+ 6.9	+ 7.9	—18.9	—13.1	—17.3	—20.3
97	— 99.9	—99.9	—99.9	—99.9				
98A	+120.0	+91.9	+83.3	+18.2				
98B	— 85.1	—88.4	—85.9	—78.8				
98C	+ 13.1	— 1.2	+ 7.4	—10.7	—14.2	+ 1.8		
101	— 46.9	—22.6	—28.2	— 5.8				
102R	+ 16.0	—18.0	— 5.0	+20.0	—27.3	—25.9	—29.8	
102C	— 97.0	—86.0	—94.0	—98.0	—99.9	—99.9	—99.9	—99.9
103	+ 13.5	+16.3	+ 9.2	+ 6.1	— 9.4	— 5.3	—21.7	
104	— 9.2	— 1.8	— 7.9	—23.7	—18.3	—92.0		
105	+ 35.0	+57.0						
106	— 11.0	—18.0						
107R	— 10.0	—12.3	—28.0	—39.1				
109	— 74.5	—68.7	—66.5	—51.9				
110	— 62.9	—55.9	—53.9	—55.3				
111	— 99.9	—99.9	—99.9	—99.9				
112	— 99.9	—99.9	—99.9	—99.9				
113	— 6.8	— 3.2	+16.6	+10.4	+ 1.4	+ 4.2		
114	— 99.9	—99.9	—99.9	—99.9				
116	— 89.6	—88.7	—90.5	—73.8				
117	— 67.2	—70.0	—72.5	—71.1				
118	— 99.9	—99.9	—99.9	—99.9				
120	— 23.6	—47.6	—33.3	—63.3				
123	+ 33.3	+16.0	+17.8	+14.5	—16.0			
124	— 99.9	—99.9	—99.9	—99.9				
129	— 99.9	—99.9	—99.9	—99.9	—49.6	—39.2	—45.1	—41.3
130	— 4.4	— 1.7	— 2.1	+10.7	+ 4.5	+24.6	— 5.8	
131	— 9.0	—32.0	—18.4	—26.2	—31.6	— 8.6		
132C	+ 1.5	+ 7.5	— 2.7	— 0.0	—24.1	—42.5	—39.5	
136	+ 30.7	+16.3	+ 6.4	—13.8				
137C	— 12.0	—16.0	— 6.0	—61.0	+30.0	+10.0	+ 3.0	—14.0
139	— 99.9	—99.9	—99.9	—99.9				
141R	+ 29.8	+10.4	+ 4.3	+ 6.3				
141Y	+ 25.1	+ 7.2	— 8.2	— 7.1	—14.4	—27.1	—20.1	
142	— 10.3	—36.5	—98.0	—98.1	—98.7	—99.7		
143	— 98.0	—98.0	—98.0	—98.0				
144	— 33.7	—15.5	— 8.5	— 9.6				
163	— 99.9	—99.9	—99.9	—99.9				
16M	— 99.0	—45.0						
63M	— 84.2	—59.9	—92.4	—83.3	+31.0	—38.0		
412M	— 91.0	—89.0	—98.0	—98.0	—98.0	—98.0		
61M	— 99.9	—99.9	—99.9	—99.9				
1G	+ 11.9	— 0.4	+39.4	+ 0.0	— 7.8			
52G	+ 31.3	— 5.8	+ 4.6	+14.4	—11.4			
71G	+ 19.0	+13.7	— 7.7	—15.5	+22.2			
76G	— 9.9	+17.2	+ 7.1	+44.2	—18.8			
6B	+ 51.8	+21.0	+ 4.4	—11.8	— 7.7			
31B	+ 20.1	—12.5	— 3.8	+26.6	+16.6			
41B	+ 7.9	— 1.4	—14.5	+17.1	+ 4.9			
48B	+ 26.7	+41.1	+10.0	— 3.0	—19.8			
1Z	+ 16.1	+ 9.0	+ 2.3	— 7.1	—13.3			
2Z	+ 31.3	— 7.7	— 3.6	+11.1	— 3.9			
H1	+ 7.1	+ 4.6	— 1.4	— 3.6	— 5.3	—18.3		
H19	+ 10.2	+ 6.1	+ 1.3	— 7.6	—10.0	+ 0.4		

plated immediately before heating and immediately after cooling. Plates were incubated at 25°C for five days. From these data the percentage decrease or increase caused by heating was determined. These percentages are given in Table 6 with the number of trials on each of the selected cultures.

The common practice was to use a 0.1 or 0.01 dilution in milk, of the original 15-hour growth. The number of bacteria present even under these conditions was usually over a million per cc sothat no data are submitted to show the influence of the dilution on the percentage of individuals killed. When a milk culture was sufficiently high in acid to coagulate upon the application of heat, the coagulum functioned as a protection for the cells against the penetration of heat. A probable explanation is the lack of mobility of the contents of the tube (Magoon and Culpepper, 1921).

In this way all cultures of which the majority thermal death point was less than 145°F for 30 minutes were eliminated. The final treatment left six cultures resembling *Streptococcus thermophilus*, six yellow sarcinae, two yellow micrococci, and nine cultures of a relatively small, dullish grey, non-motile, Gram-positive rod. In the case of several determinations of the percentage survival of the rods, a percentage increase ranging from 10 to 120 was obtained. Apparently, in some cases, the species had grown and in others the bacterial clumps had disintegrated during the heat treatment. Repeated experiments showed that no error had been made in diluting the samples or in marking the plates.

EFFECT OF HEAT ON DISINTEGRATION OF BACTERIAL CLUMPS

The following cultures listed in Table 6 were chosen for further study because of their peculiar resistance. These cultures were grouped under four types according to the similarity of the characters displayed and in reality represent four species. Type I consisted of cultures Nos. 17, 20R, 75, 87, 98A, 113, 130, 136, and 141R; Type II of cultures Nos. 18, 23R, 35, 72, 103, and 123; Type III of cultures Nos. 19, 20Y, 70, 89, 96+, and 141Y; and Type IV of cultures Nos. 98C and 132. The sources of these cultures were as follows: Nos. 17 and 18 from Jackson, Mich.; Nos. 19, 20R, 20Y, 23R, 35, 87, 89, 96+, 98A, 98C, 103, 113, and 123 from Kansas City, Mo.; Nos. 70 and 72 from Boston; No. 75 from Milwaukee; and Nos. 130, 132, 136, 141R, and 141Y from Baltimore.

TABLE 7.—INFLUENCE OF HEAT ON THE DISINTEGRATION OF THE BACTERIAL CLUMP IN MILK CULTURES.

TYPE	CULTURE No.	AVERAGE SIZE OF CLUMP IN MILK CULTURE*							
		Unheated	Heated at 62.8°C for						
			10 minutes	20 minutes	30 minutes		60 minutes	180 minutes	
					Size	Ratio $\frac{U†}{H}$		Size	Ratio $\frac{U†}{H}$
I	21	2.49			2.08	1.19		2.04	1.22
	98A	2.43			2.24	1.09		2.09	1.16
	98A	2.59			2.14	1.21		2.09	1.24
	104	2.00			2.03	0.98		1.88	1.07
	113	3.88			2.66	1.46		1.85	2.10
	130	2.56			2.04	1.25		1.88	1.36
	136	2.24			2.20	1.02		2.15	1.04
Average						1.17			1.31
II	23R	2.65			2.28	1.17		2.08	1.27
	35	2.92			2.15	1.36		1.89	1.55
	72	2.62			2.28	1.15		2.12	1.24
	103	2.00			1.85	1.07		1.78	1.12
	123	1.84			1.65	1.12		1.48	1.24
Average						1.17			1.28
III	1Z	36.7	28.8	32.4	28.4	1.29		22.7	1.61
	1Z	59.9	53.6	41.4	40.2	1.49	29.0	27.4	2.18
	2Z	78.2	36.5	43.1	23.1	3.38	11.5	21.2	3.69
	2Z	29.0			26.0	1.12			
	19	3.0			2.5	1.20		2.0	1.50
	19	4.4			4.2	1.05		3.7	1.16
	20	8.4			5.9	1.42		6.5	1.30
	70	13.9			11.5	1.21		10.4	1.34
	95	8.3			5.8	1.43		5.4	1.54
	96+	8.1			6.5	1.25		5.2	1.56
Average						1.48			1.76

*Number of individual cells divided by the number of clumps observed gives the average number of cells per clump.

†Ratio $\frac{U}{H} = \frac{\text{Size of clump in unheated milk}}{\text{Size of clump after heating}}$.

The attempt to explain the results by assuming that the clumps were broken up sufficiently to cause the entire percentage increase is unsatisfactory in the case of Types I and II. Nearly half as many more clumps appeared in the case of Type III at the end of heating 30 minutes at 62.8°C as at the beginning. Table 7 shows the customary breaking up of the clumps of bacteria after the milk had been subjected to pasteurization temperatures for different periods of time. On several occasions members of Type I lived for four and five days at 62.8°C.

SURVIVAL AT PASTEURIZING TEMPERATURES

In order to determine how long species from these four types would survive in milk cultures at pasteurizing temperatures, the following technic was used. Cultures were first inoculated into milk and allowed to incubate at 25°C for 18 hours when they were placed at 62.8°C in a water bath. At different times, ranging between 15 and 240 minutes, a loopful of the milk culture was streaked on an agar slant which was then incubated at 25°C. At the end of seven days the amount of the growth was recorded. Table 8 demonstrates clearly that Types I and III survived better than Types II and IV. Altho the technic employed was not above criticism, it indicates the relative ability of the different species to survive at the pasteurizing temperature.

CULTURES FROM GENEVA, N. Y., AND BURLINGTON, VT., MILK SUPPLIES

The classification of the 132 cultures from the Geneva milk supply is based on a knowledge of the following characters: (1) Morphology, as determined by the Gram stain of the agar slant culture and by the characteristic clumping of the bacteria in the milk culture; (2) chromogenesis on agar slants; (3) litmus milk reactions; and (4) acid production from d-glucose, lactose, and sucrose. Indicators were used to determine the approximate hydrogen-ion concentration of the sugar broths after incubating the cultures for 10 days at 25°C. The above determinations were run twice in duplicate within a period of 12 months. The ability to ferment glycerol was determined during the second series of tests. The same procedure was employed for classifying the 41 cultures from the Burlington milk supply. Nearly all of the 35 coccus cultures from Geneva and the 7 from Burlington were yellow sarcinae. No further tests were run on these cultures as it was assumed that they were identical with the other members of Type III.

TABLE 8.—SURVIVAL OF MILK CULTURES WHEN HEATED AT 62.8°C FOR VARYING PERIODS OF TIME.*

PERIOD OF HEATING IN MINUTES	CULTURE NO., TYPE I								CULTURE NO., TYPE II					
	17	20R	75	87	98A	98A	113	130	18	23R	35	72	103	132
15	—	F	A	—	A	V	T	A	—	T	—	O	F	T
20	V	—	—	V	—	—	—	—	V	—	F	—	—	—
30	V	F	F	V	V	V	T	A	A	T	A	F	O	O
60	V	A	T	V	V	V	T	V	A	T	A	F	O	O
90	—	F	T	—	V	A	T	A	—	O	—	O	—	—
120	V	F	T	V	V	V	O	V	T	O	T	O	O	O
180	V	F	O	V	V	V	O	A	F	O	O	O	O	O
240	—	T	O	—	V	V	T	V	—	O	—	O	—	—

	CULTURE NO., TYPE III									CULTURE NO., TYPE IV	
	19	20Y	70	89	96+	141Y	1Z	2Z	95	98C	132
15	V	V	—	—	—	F	—	—	—	F	—
20	—	—	V	V	V	—	V	V	F	—	F
30	V	V	V	V	V	F	V	A	F	T	A
60	V	V	V	V	V	F	V	V	F	O	A
90	V	V	—	—	—	T	—	—	—	O	—
120	V	V	A	V	V	O	A	T	T	O	T
180	O	O	V	T	V	O	T	A	O	O	O
240	O	O	—	—	—	O	—	—	—	O	—

*The symbols indicate as follows:
— = No test
O = No growth
T = Trace of growth
F = Fair growth
A = Abundant growth
V = Very abundant growth

The 97 rods from Geneva and the 34 from Burlington were identical and are considered as members of Type I. To present the data in detail is unnecessary. In Table 6 are included the percentages of increase or decrease in the plate counts of four typical cultures from each source when milk cultures of these species are subjected to pasteurizing temperatures.

CULTURES FROM MISCELLANEOUS SOURCES

Similar data are included in Table 6 to show the percentage survival of cultures 1Z and 2Z (accidental isolations) and cultures H1 and H19 (isolations from plates sent to the Geneva laboratory by H. G. Harding).

CULTURES FROM MILKING MACHINES

Milk cultures of the 415 cultures isolated from milking machines were subjected to pasteurization at different temperatures. Some of

TABLE 9.—RESULTS OF PASTEURIZING 159 OF THE 415 CULTURES FROM MILKING MACHINES.

CULTURE No.	MILK CULTURES PASTEURIZED FOR 30 MINUTES IN												APPROXIMATE INDIVIDUAL MICROSCOPIC COUNT IN MILLIONS PER CC
	Test tubes at								Sternberg bulbs at				
	145°F		150°F		155°F		160°F		142°F		145°F		
	Number of trials	Number of survivals	Number of trials	Number of survivals	Number of trials	Number of survivals	Number of trials	Number of survivals	Number of trials	Number of survivals	Number of trials	Number of survivals	
1	6	3	3	1	4	1	4	2	1	0	2	0	20.0
4	6	2	3	0	2	1	2	0	1	0	2	0	3.0
5	7	4	4	2	2	2	4	4	1	0	2	0	7.0
16	5	1	3	0	2	0	2	0	1	0	2	0	1.0
18	6	3	3	1	2	2	2	0	1	0	2	0	1.0
22	3	2	4	2	2	1	2	0	1	0	2	0	2.0
32	6	3	3	3	2	2	2	2	1	0	2	0	2.0
34	4	1	1	0	–	–	–	–	1	0	2	0	25.0
46	6	4	5	3	4	2	4	0	1	0	2	0	3.0
47	6	3	3	3	2	2	4	2	1	0	2	0	10.0
63	7	5	4	4	2	0	4	2	1	1	2	2	4.0
70	4	1	–	–	–	–	–	–	1	0	2	0	20.0
71	3	0	–	–	–	–	–	–	1	0	2	0	30.0
72	3	0	–	–	–	–	–	–	1	0	2	0	12.0
73	3	0	–	–	–	–	–	–	1	0	2	0	20.0
74	2	0	–	–	–	–	–	–	1	0	2	0	18.0
76	7	7	6	3	2	2	4	2	1	0	2	0	300.0
76	–	–	–	–	–	–	–	–	1	0	2	0	2.0
77	5	1	3	3	2	2	4	2	1	0	2	0	1.0
78	2	0	1	–	–	–	–	–	1	0	2	0	15.0
79	2	0	–	–	–	–	–	–	1	0	2	0	16.0
81	6	3	4	3	2	0	4	2	1	0	2	0	1.0
83	5	2	3	1	2	1	2	0	1	1	2	2?	65.0
84	2	0	–	–	–	–	–	–	1	0	2	0	10.0

85	5	3	3	2	2	2	4	0	1	0	2	0	2.0
86	2	0	–	–	–	–	–	–	1	0	2	0	30.0
87	2	0	–	–	–	–	–	–	1	0	2	0	5.0
88	2	0	–	–	–	–	–	–	1	0	2	0	45.0
89	2	0	–	–	–	–	–	–	1	0	2	0	2.0
90	5	2	3	1	2	0	2	0	1	0	2	0	0.3
91	5	1	3	3	2	1	2	0	1	0	2	0	200.0
91	–	–	–	–	–	–	–	–	1	1?	2	0	10.0
94	6	5	4	3	2	0	2	0	1	1	2	2	15.0
96	6	5	4	2	2	2	4	4	1	1	2	1	40.0
96	–	–	–	–	–	–	–	–	1	0	2	0	8.0
98	2	0	–	–	–	–	–	–	1	1	2	2	8.0
99	7	7	4	4	2	2	4	3	1	1	2	2	210.0
99	–	–	–	–	–	–	–	–	1	1	2	2	8.0
100	7	7	2	2	2	2	4	4	1	1	2	2	300.0
100	–	–	–	–	–	–	–	–	1	1	2	2	2.0
101	6	5	4	4	2	2	4	4	1	0	2	0	12.0
102	7	7	2	1	2	1	2	0	1	0	2	0	1.0
103	2	1	–	–	–	–	–	–	1	0	2	0	4.0
104	7	4	4	4	2	2	4	3	1	0	2	0	4.0
105	7	7	2	2	2	2	4	3	1	0	2	0	1.0
106	7	7	2	2	2	2	4	4	1	1	2	2	40.0
106	–	–	–	–	–	–	–	–	1	1	2	2	3.0
110	8	8	3	3	2	1	4	4	1	1	2	2	140.0
110	–	–	–	–	–	–	–	–	1	1	2	2	5.0
111	8	8	3	3	2	2	4	3	1	1	2	2	400.0
111	–	–	–	–	–	–	–	–	1	1	2	2	10.0
112	5	3	3	0	2	1	4	4	1	0	2	0	1.0
113	2	0	–	–	–	–	–	–	1	0	2	0	0.3
116	2	0	–	–	–	–	–	–	1	0	2	0	17.0
117	6	6	5	5	2	2	4	1	1	1	2	2	10.0
120	–	–	–	–	–	–	–	–	1	1	2	2	1.0
124	6	6	3	2	2	0	4	1	1	1	2	2	4.0
126	6	5	3	3	2	2	5	4	1	1	2	2	200.0
126	–	–	–	–	–	–	–	–	1	1	2	2	10.0

TABLE 9.—*Continued.*

Culture No.	Milk cultures pasteurized for 30 minutes in												Approximate individual microscopic count in millions per cc
	Test tubes at								Sternberg bulbs at				
	145°F		150°F		155°F		160°F		142°F		145°F		
	Number of trials	Number of survivals	Number of trials	Number of survivals	Number of trials	Number of survivals	Number of trials	Number of survivals	Number of trials	Number of survivals	Number of trials	Number of survivals	
127	2	0	–	–	–	–	–	–	1	0	2	0	3.0
128	2	0	–	–	–	–	–	–	1	0	2	0	10.0
129	8	6	5	4	4	4	4	3	1	1	2	2	25.0
130	8	3	6	1	4	0	4	1	1	0	2	0	10.0
137	4	2	4	2	2	1	4	4	1	0	2	0	1.0
141	6	4	3	2	2	1	4	1	1	0	2	0	4.0
142	4	1	2	0	–	–	2	0	1	0	2	0	7.0
142A	6	6	3	3	1	0	2	1	1	1	2	2	400.0
142A	–	–	–	–	–	–	–	–	1	1	2	2	3.0
143	5	3	3	1	2	2	2	1	1	1	2	2	300.0
143	–	–	–	–	–	–	–	–	1	1	2	2	3.0
143A	8	7	5	3	2	2	2	2	1	1	2	2	15.0
145A	3	3	3	3	2	2	2	2	1	1	2	2	3.0
147	5	3	3	3	2	2	2	2	1	1	2	2	2.0
148	6	6	3	3	2	2	2	2	1	1	2	2	3.0
154A	6	6	3	2	2	2	2	1	1	0	2	0	1.0
155	6	6	4	3	2	2	2	2	1	1	2	2	4.0
157	6	6	3	3	2	2	2	2	1	1	2	2	2.0
158	–	–	–	–	–	–	–	–	1	1	2	2	5.0
158	6	6	3	3	2	2	2	2	1	1	2	2	300.0
159	6	6	3	3	2	2	2	2	1	1	2	2	2.0
161	5	3	2	0	–	–	–	–	1	1	2	2	1.0
164	5	5	3	3	2	2	2	2	1	0	2	0	30.0
165	5	4	4	4	2	2	2	2	1	0	2	0	2.0

166	6	1	3	3	2	1	2	2	1	0	2	0	1.0
167	5	5	6	4	2	0	2	1	1	0	2	0	3.0
169	3	3	–	–	–	–	–	–	1	0	2	0	3.0
170	5	5	2	0	1	1	2	2	1	0	2	0	4.0
172	5	3	3	2	2	1	2	1	1	0	2	0	20.0
173	2	1	–	–	–	–	–	–	1	0	2	0	7.0
180	3	3	3	3	2	2	2	2	1	0	2	0	0.2
181	1	1	3	2	2	2	2	1	1	0	2	0	15.0
183	5	4	5	2	2	0	2	0	1	0	2	0	10.0
185	2	0	–	–	–	–	–	–	1	0	2	0	40.0
190	4	2	2	0	–	–	–	–	1	0	2	0	4.0
192	2	0	–	–	–	–	–	–	1	0	2	0	20.0
196	2	2	–	–	–	–	–	–	1	0	2	0	30.0
205	6	6	4	4	2	2	2	2	1	1	2	2	100.0
206	4	4	2	2	–	–	–	–	1	0	2	0	1.0
215	6	5	4	4	2	2	2	2	1	1	2	2	1.0
226	6	6	4	3	2	2	2	0	1	1	2	2	5.0
228	6	3	4	2	2	2	2	1	1	0	2	0	17.0
229	3	3	2	1	–	–	–	–	1	0	2	0	2.0
234	6	4	4	4	2	1	2	0	1	0	2	0	10.0
266	4	2	4	1	2	0	2	0	1	0	2	0	5.0
268	3	3	2	0	2	2	2	1	1	1	2	2	60.0
268	–	–	–	–	–	–	–	–	1	1	2	2	10.0
269	5	4	3	1	2	1	2	1	1	1	2	2	20.0
270	3	3	3	2	2	1	2	1	1	1	2	2	10.0
304	6	4	4	1	2	0	2	0	1	0	2	0	20.0
331	3	3	3	2	2	1	2	2	1	1	2	2	20.0
331	–	–	–	–	–	–	–	–	1	1	2	2	30.0
352	6	6	4	4	2	2	2	2	1	0	2	0	4.0
353	1	0	1	0	1	0	2	0	1	0	2	0	2.0
359	4	2	2	0	1	0	–	–	1	0	2	0	1.0
369	6	6	4	2	2	0	2	1	1	1	2	2	25.0
372	3	1	3	1	2	0	2	0	1	1	2	2	3.0
374	4	2	2	1	–	–	–	–	1	0	2	0	20.0
375	6	5	4	3	2	1	2	0	1	0	2	0	4.0

TABLE 9.—*Concluded.*

CULTURE No.	MILK CULTURES PASTEURIZED FOR 30 MINUTES IN												APPROXIMATE INDIVIDUAL MICROSCOPIC COUNT IN MILLIONS PER CC
	Test tubes at								Sternberg bulbs at				
	145°F		150°F		155°F		160°F		142°F		145°F		
	Number of trials	Number of survivals	Number of trials	Number of survivals	Number of trials	Number of survivals	Number of trials	Number of survivals	Number of trials	Number of survivals	Number of trials	Number of survivals	
378	4	3	1	1	–	–	–	–	1	0	2	0	4.0
379	3	1	3	2	2	0	2	1	1	0	2	0	2.0
390	3	2	3	1	2	1	2	0	1	0	2	0	3.0
409	6	5	4	3	2	2	2	0	1	0	2	0	3.0
411	4	3	2	1	–	–	–	–	1	1	2	2	10.0
412	3	2	2	1	2	1	2	0	1	1	2	2	10.0
417	–	–	2	1	–	–	2	0	1	0	2	0	2.0
418	–	–	2	1	–	–	2	0	1	0	2	0	2.0
427	–	–	2	0	–	–	2	1	1	0	2	0	16.0
428	–	–	2	1	–	–	2	0	1	0	2	0	20.0
430	–	–	2	1	–	–	2	0	1	0	2	0	——
431	–	–	2	0	–	–	2	1	1	0	2	0	——
434	–	–	2	1	–	–	2	0	1	0	2	0	——
437	–	–	2	0	–	–	2	1	1	0	2	0	——
441	–	–	2	0	–	–	2	1	1	0	2	0	——
443Y	–	–	2	0	–	–	2	1	1	0	2	0	——
444	–	–	2	2	–	–	2	1	1	0	2	0	——
448	–	–	2	1	–	–	2	1	1	0	2	0	——
451	–	–	2	1	–	–	2	1	1	1	2	2	3.0
452	–	–	2	2	–	–	2	1	1	0	2	0	——
453	–	–	2	0	–	–	2	2	1	0	2	0	——
454	–	–	2	0	–	–	2	1	1	0	2	0	6.0
455A	–	–	2	1	–	–	2	0	1	0	2	0	30.0
469	–	–	2	0	–	–	2	1	1	0	2	0	15.0

470	–	–	2	1	–	–	2	0	1	0	2	0	30.0
471	–	–	2	1	–	–	2	0	1	0	2	0	30.0
475	–	–	2	2	–	–	2	0	1	0	2	0	4.0
477	–	–	2	2	–	–	2	1	1	0	2	0	2.0
478	–	–	2	1	–	–	2	0	1	0	2	0	20.0
480	–	–	2	1	–	–	2	0	1	0	2	0	50.0
485	–	–	2	1	–	–	2	0	1	0	2	0	3.0
489A	–	–	2	0	–	–	2	2	1	0	2	0	2.0
493	–	–	2	0	–	–	2	1	1	0	2	0	10.0
495	–	–	2	0	–	–	2	1	1	0	2	0	6.0
497	–	–	2	1	–	–	2	1	1	0	2	0	2.0
512	–	–	2	1	–	–	2	1	1	0	2	0	8.0
522	–	–	2	0	–	–	2	1	1	0	2	0	2.0
524	–	–	2	0	–	–	2	2	1	0	2	0	25.0
534	–	–	2	0	–	–	2	1	1	0	2	0	30.0
548	–	–	2	1	–	–	2	0	1	0	2	0	——
553	–	–	2	1	–	–	2	1	1	0	2	0	6.0
576	–	–	2	1	–	–	2	1	1	0	2	0	10.0
579	–	–	2	2	–	–	2	0	1	0	2	0	——
580	–	–	2	2	–	–	2	2	1	0	2	0	10.0
583	–	–	2	2	–	–	2	2	1	0	2	0	4.0
584	–	–	2	0	–	–	2	1	1	0	2	0	4.0
594	–	–	2	2	–	–	2	2	1	1	2	2	3.0
631	–	–	2	1	–	–	2	0	1	0	2	0	2.0
637	–	–	2	1	–	–	2	0	1	0	2	0	——
639	–	–	2	1	–	–	2	0	1	0	2	0	——
650	–	–	2	2	–	–	2	0	1	0	2	0	4.0
609	–	–	2	0	–	–	2	0	1	1	2	2	3.0
663	–	–	2	2	–	–	2	0	1	0	2	0	3.0
670	–	–	2	1	–	–	2	1	1	0	2	0	——

TABLE 10.—CHARACTERS OF THE 42 HEAT-RESISTANT MILKING MACHINE CULTURES WHICH SURVIVED PASTEURIZATION IN STERNBERG BULBS.*

CULTURE No.	MORPHOLOGY	CHROMOGENESIS	GRAM STAIN	AEROBE	GELATIN LIQUEFACTION	NITRATE REDUCTION	FERMENTATION OF			MILK REACTIONS			USE OF AMMONIA NITROGEN
							D-glucose	Lactose	Sucrose	Acid production	Acid production and coagulation	Peptonization	
63	C	y	+	+	+	−	−	−	−	+	−	−	−
83	C	w	−	+	+	+	+	+	+	−	−	−	?
94	C	y	+	+	+	+	+	+	−	+	+	−	+
96	C	y	+	+	+	+	+	+	+	+	+	+	+
98	C	w	+	+	−	+	+	+	+	+	−	−	?
99	R	y	+	+	+	+	+	+	−	+	+	+	?
100	R	y	+	+	+	−	+	+	−	+	+	+	?
106	C	y	+	+	+	−	+	+	+	+	+	+	?
110	R	y	+	+	+	+	+	+	−	+	+	+	?
111	R	y	+	+	+	−	+	+	−	+	+	+	?
117	C	y	+	+	+	−	+	+	+	+	−	−	?
120	C	w	+	+	+	−	+	+	−	+	+	+	−
124	C	y	+	+	+	+	+	+	−	+	+	−	+
126	C	y	+	+	−	−	+	−	−	+	+	−	−
129	C	w	+	+	−	−	+	+	+	+	+	−	?
142A	R	y	+	+	+	−	+	+	−	+	+	+	?

143	R	y	+	+	+	—	+	+	—	+	+	+	?
143A	C	y	+	+	—	+	+	+	+	+	+	—	+
145A	R	w	+	+	—	—	+	+	+	+	—	—	?
147	R	w	+	+	—	—	+	+	+	+	—	—	—
148	R	w	+	+	—	—	+	+	+	+	—	—	?
155	C	w	+	+	—	—	+	+	+	+	—	—	?
157	R	w	+	+	—	+	+	+	+	+	+	—	?
158	R	y	+	+	+	—	+	+	—	+	+	—	?
159	C	w	+	+	—	+	+	+	+	+	—	—	?
161	C	w	+	+	—	—	+	+	+	+	—	—	?
205	C	y	+	+	+	+	+	+	—	+	—	—	?
215	R	w	+	+	+	—	+	+	+	+	+	—	?
226	C	y	+	+	—	+	+	+	—	+	+	—	?
268	C	y	+	+	+	+	+	+	+	+	—	—	+
269	C	y	+	+	+	+	+	+	+	+	—	—	+
270	C	w	+	+	+	+	+	+	+	+	+	—	?
331	C	y	+	+	+	—	+	+	—	+	+	—	—
340	R	w	—	+	—	+	+	+	+	+	—	—	?
369	C	y	+	+	—	—	+	+	—	+	—	—	+
372	C	y	+	+	—	—	+	+	—	+	+	—	?
373	C	y	+	+	+	+	+	+	—	+	—	—	+
411	C	y	+	+	+	+	+	+	—	+	+	—	+
412	R	y	+	+	+	+	+	+	+	+	+	+	?
451	R	y	+	+	+	—	—	—	—	—	+	+	?
594	C	y	+	+	+	+	+	+	+	+	—	—	+
609	C	w	+	+	—	—	+	+	+	+	+	—	?

*The symbols indicate as follows:

C—Coccus form
R—Rod form

w—white
y—yellow

the cultures were pasteurized in cotton-stoppered test tubes at 145°, 150°, 155°, and 160°F for 30 minutes and cooled to less than 40°F for 20 minutes. Agar slants were made from the milk tubes and incubated at 25°C for seven days. The amount and the distribution of the growth indicates in a limited manner the numbers of bacteria surviving the action of the heat. The milk cultures also were incubated for seven days when microscopic preparations were made to determine if growth had occurred. For comparison other similar data are submitted based on heating the same cultures in Sternberg bulbs at 142° and 145°C. After the desired heating and cooling the contents of the Sternberg bulbs were plated immediately. A partial quantitative determination of the percentage killed can be obtained in this manner. Only the results from heating 159 of the 415 cultures are presented in Table 9, as they are the only ones where the open tube treatment and Sternberg bulb method of heating could be compared. A greater number of the cells were killed in the Sternberg bulbs than when the same culture was heated in test tubes. Brown and Peiser (1916) argue that no advantage is obtained when the Sternberg bulb is used.

Only 42 cultures from milking machines among the 415 were able to survive pasteurization. A more complete description of these cultures appears in Table 10.

Studies to determine the percentage decrease or increase of the living bacteria in the milk cultures of the 42 cultures revealed nothing unusual. In all cases, except the yellow cocci and the three non-nitrate-reducing, non-liquefying rods, more than half of the cells were killed. At least twice as many yellow coccus cultures as cultures

TABLE 11.—DISTRIBUTION OF THE 42 HEAT-RESISTANT MILKING MACHINE CULTURES WHEN GROUPED ACCORDING TO MORPHOLOGY, CHROMOGENESIS, GELATIN LIQUEFACTION, AND NITRATE REDUCTION.

MORPHOLOGY AND CHROMOGENESIS	GELATIN LIQUEFACTION				TOTAL NUMBER OF CULTURES
	Positive		Negative		
	Positive nitrate reduction	Negative nitrate reduction	Positive nitrate reduction	Negative nitrate reduction	
Coccus, yellow	10	5	1	2	18
Coccus, white	2	3	1	3	9
Rod, yellow	3	4	1	0	8
Rod, white	0	2	1	3	6
Cultures resembling *Streptococcus lactis*	0	0	0	1	1

of any other one type survived. Further study showed that many of these were sarcinae. Table 11 contains a summary of the characters of the 42 cultures under discussion. All except 3 of the 42 heat-resistant species came from milking machines subjected to sterilization by chemicals, chiefly hypochlorites. Nearly two-thirds of these cultures were cocci. The data suggest that heat-resistant bacteria are more likely to be present in milk where chemical sterilization of milking machines is employed.

DESCRIPTION OF TYPES ISOLATED

QUALITATIVE STUDIES

The ability of 28 cultures to use certain of the carbohydrates, organic acids, amino acid nitrogen, and certain nitrogenous compounds has been determined. In a few cases where the cost of the test substances was prohibitive, the ability to use the substances was determined on only a few of the cultures from each type. These 28 cultures are distributed as follows: 10 in Type I, 6 in Type II, 8 in Type III, 2 in Type IV, and 2 in Type V. Because of the necessity of many repetitions, only a summary table (Table 12) indicating the final pH after ten days' incubation at 30°C is presented. No change in pH may indicate failure to grow or failure to liberate acid or alkali from the fermentable substance. An increase in alkalinity in broths containing organic acids occurs when the organic acid is utilized which liberates the alkali used to neutralize the broth. For the methods used, the reader is referred to the section under technic (page 14).

Type I may or may not ferment the pentoses and trisaccharides (raffinose); usually ferments the hexoses, disaccharides, glucosides, complex carbohydrates, and hexose and pentose alcohols; and generally fails to utilize the organic acids and amino acids. Hippuric acid is available.

Type II fails to ferment the pentoses and the amino acids; usually ferments the hexoses, disaccharides, trisaccharides, complex carbohydrates, and glucosides. Some of the hexose alcohols and glycerol are available, while the pentose alcohols are not. Some of the organic acids are utilized.

Type III ferments the pentoses, hexoses, disaccharides, trisaccharides, glucosides, and pentose and hexose alcohols. Some of the organic acids and amino acids are utilized.

TABLE 12.—UTILIZATION OF AND AVERAGE FINAL pH IN MEDIA CONTAINING VARIOUS FERMENTABLE SUBSTANCES AFTER GROWTH OF CULTURES FOR TEN DAYS AT 30°C.

FERMENTABLE SUBSTANCE	TYPES									
	I		II		III		IV		V	
	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth
Nutrient broth	7.0	+	7.0	+	7.3	+	7.0	+	7.2	+
Pentoses										
Arabinose	5.2	±	7.0	—	8.1	+	5.0	+	7.6	+
Xylose	6.0	±	7.0	—	7.4	+	5.7	+	7.5	+
Hexoses										
D-glucose	4.9	+	5.0	+	7.6	+	5.3	+	7.5	+
Fructose	5.3	+	5.5	+	7.5	+	5.4	+	7.1	+
Mannose	5.1	+	5.1	+	7.4	+	5.6	+	7.7	+
Galactose	5.6	+	5.6	+	7.9	+	5.4	+	5.4	+
Disaccharides										
Lactose	5.1	+	5.0	+	7.5	+	5.1	+	7.4	+
Sucrose	5.3	+	5.5	+	8.1	+	5.3	+	7.5	+
Trisaccharide										
Raffinose	5.6	±	5.3	+	8.3	+	5.9	+	7.4	+
Glucosides										
Amygdalin	6.3	+	5.2	+	7.6	+	5.6	+	7.5	+
Salicin	5.2	+	4.7	+	7.9	+	5.1	+	7.0	?
Complex carbohydrates										
Inulin	7.2	+	7.4	+	8.3	+	5.8	+	7.4	+
Dextrin	6.6	+	6.1	+	—	+	6.0	+	6.7	+
Soluble starch	5.8	+	7.6	+	7.9	+	6.2	+	7.2	+

Alcohols										
Mannite	5.8	+	6.3	+	8.3	+	7.0	?	6.8	+
Dulcite	6.2	+	7.0	—	7.6	+	6.5	+	6.7	+
Sorbite	6.6	+	7.2	+	7.8	+	7.6	+	7.7	+
Adonite	7.6	±	7.0	—	7.6	+	7.4	+	7.6	+
Erythrite	6.4	±	7.0	—	7.7	+	7.4	+	6.6	+
Glycerol	6.0	+	5.2	+	0.8	+	5.5	+	7.9	+
Organic acids										
Oxalic	7.0	—	7.0	—	7.0	—	7.0	—	7.0	—
Malonic	—	?	8.0	+	7.5	+	—	?	—	?
Succinic	7.0	—	7.4	+	7.6	+	7.4	+	7.0	—
Glycolic	7.0	—	7.0	—	7.0	—	7.0	—	7.0	—
Lactic	7.0	—	7.0	—	7.0	—	7.0	—	7.0	—
Malic	—	?	8.1	+	8.0	+	7.4	+	7.0	—
Tartaric	7.0	—	7.0	—	7.0	—	7.0	—	7.0	—
Citric	7.0	—	7.0	—	7.9	+	7.0	—	7.0	—
Oleic	7.0	—	7.0	—	7.0	—	7.0	—	7.0	—
Fumaric	7.0	—	7.4	+	7.0	—	7.0	—	7.0	?
Maleic	7.0	—	7.0	?	7.0	—	7.0	—	7.0	—
Hippuric	6.3	+	7.5	+	7.5	+	6.4	+	7.4	+
Amino acids										
Glycocoll	7.0	—	7.0	—	5.6	+	7.0	—	7.0	—
Glutamic acid	7.0	—	7.0	—	5.8	+	7.0	—	7.2	+
Aspartic acid	7.0	—	7.0	—	7.0	—	7.0	—	7.4	+
Miscellaneous										
Uric acid	7.0	—	7.0	—	7.0	—	7.0	—	7.0	—
Nucleic acid	7.0	—	7.0	—	7.0	—	7.0	—	7.0	—
Urea	7.0	—	7.0	—	7.0	—	7.0	—	7.0	—
Lactose peptone bile	7.0	+	7.0	+	7.0	+	7.0	+	7.0	+
Sodium asparaginate	7.0	—	7.0	—	7.0	—	7.0	—	7.0	—
Ammonium citrate	7.0	—	7.0	—	7.0	—	7.0	—	7.0	—

Type IV ferments the pentoses, hexoses, disaccharides, trisaccharides, glucosides, and pentose and hexose alcohols. Succinic, malic, and hippuric acids are utilized. The amino acids are not available.

Type V ferments the pentoses, hexoses, disaccharides, trisaccharides, glucosides, and pentose and hexose alcohols. Hippuric and some of the amino acids are fermented.

Uric acid, nucleic acid, urea, sodium asparaginate, and ammonium citrate are not available as food for any of these types. Growth occurs in lactose peptone bile, altho there is no change in the pH.

QUANTITATIVE TESTS

Since the types of organisms were isolated from a product widely used for human consumption, further studies have been conducted to determine some of the quantitative changes in (1) the amount of sugars (d-glucose, lactose, and sucrose) used by representative species from each of the five types. Nutrient broth containing 1 per cent of the fermentable substance was used. Determinations were also made of the lactose used when the cultures were growing in milk. Calcium carbonate has been added to the solutions in some cases. Studies on the production of (2) ammonia and (3) amino acid nitrogen have been completed when the cultures were grown in the above solutions. The amount of (4) the volatile acid produced in two weeks and (5) the development of titratable acidity have been determined. Alcohol and aldehyde tests were applied to the first 10-cc portions of the volatile acid distillate. The results of the above tests appear in Tables 13, 14, 15, 16, and 17.

METHODS

The sugar determinations were made according to the Bertrand (1906) method. The lactose in milk was determined by adding to 5 cc of milk in a 100-cc volumetric flask, 2 cc of a copper sulfate solution (34.639 grams of $CuSO_4 \cdot 5H_2O$ in 500 cc of distilled water) and 2 cc of a 0.5 N solution of sodium hydroxide. When the flasks were half filled with water the contents were thoroly shaken. The flasks were filled to the mark with water and the precipitated proteins were filtered off. Lactose determinations were made of the filtrate according to the Bertrand (1906) method.

Ammonia nitrogen determinations were made by the Folin (1902) method. Sodium chloride was used in all determinations instead of sodium carbonate.

Amino acid nitrogen was determined according to the principles of the 'B method' of the original Sörensen (1908) test with partial modifications according to Brown (1923) and Levine and Carpenter (1923). Because of the small amount of amino acid nitrogen present, a 0.01N sodium hydroxide solution was employed for the titrations.

Volatile acid production was determined by the common steam distillation method from 1,000 cc of distillate. The presence or absence of alcohol in the first 10 cc of distillate was determined by the chromic acid method. The presence or absence of aldehyde and alcohol was determined by the iodoform test. All distillates gave negative results.

Type II attacks the carbohydrates actively. Type I is a little less active than Type II, while the remaining three Types attack them still more slowly. The nitrogenous compounds are more available to the species representing Types III and V than the carbohydrates in many instances. The titratable acidity increases with the amount of the carbohydrate utilized.

There was no appreciable change in the ammonia nitrogen content. Types I, II, and IV do not alter the amino acid nitrogen content. Type III shows a small increase and Type V shows just a slight increase.

The volatile acid production was detectable in Type IV. Type III produced a small amount, while Types I, II, and V failed to produce measurable amounts of volatile acids.

SPECIES NAME ASSOCIATED WITH TYPES DESCRIBED

TYPE I—*Microbacterium lacticum* ORLA-JENSEN, 1919

The size of this rod, together with its other characters, suggests the probable identity of this type with Orla-Jensen's *Microbacterium lacticum* (1919). The writer is not convinced, however, that Ayers and Johnson's *Lactobacillus thermophilus* (1924b) may not be the same species also.

Altho this species has been isolated from raw milk when the latter has been held at pasteurizing temperatures for several hours and from pasteurized milk plates, growth in sterilized skimmilk at pasteurizing temperatures when inoculated with laboratory strains has not been observed. The laboratory strains have been grown on agar slants for a period of more than a year, it being necessary to transfer them to new media about every four months. Before making

TABLE 13.—MILLIGRAMS OF SUGAR AT THE BEGINNING AND THE AMOUNTS USED UP AT THE END OF STATED PERIODS IN SOLUTIONS IN WHICH THE FIVE TYPES OF HEAT-RESISTANT BACTERIA ARE GROWING.

Type No.	Culture No.	D-glucose broth					Lactose broth					Sucrose broth					Skimmilk				
		0 days	3 days	7 days	15 days	30 days	0 days	3 days	7 days	15 days	30 days	0 days	3 days	7 days	15 days	30 days	0 days	3 days	7 days	15 days	30 days
I	1G	1,008	16	35	75	69	987	20	63	51	39	1,012	79	114	124	140	4,562	297	376	539	731
	1G	842	43	49	59	80	1,083	18	33	23	62	985	67	81	104	93	4,649	288	346	600	503
	98A	1,008	197	281	263	269	957	74	60	84	80	1,012	91	97	97	97	4,562	453	347	598	608
	98A	842	82	100	118	118	987	49	68	94	82	985	43	49	67	77	4,649	384	404	493	578
	52G	——	—	—	—	—	——	—	—	—	—	——	—	—	—	—	4,159	231	308	433	465
	52G	——	—	—	—	—	——	—	—	—	—	——	—	—	—	—	4,159	105	231	243	338
	130	——	—	—	—	—	——	—	—	—	—	——	—	—	—	—	4,159	58	172	413	338
	130	——	—	—	—	—	——	—	—	—	—	——	—	—	—	—	4,159	152	192	279	433
	136	976	34	69	87	93	957	33	38	60	52	970	49	66	78	66	4,524	221	368	491	406
	136	842	20	40	40	46	987	30	58	90	99	985	25	40	49	67	4,562	134	297	453	376
II	23R	1,008	126	197	222	222	1,083	86	154	164	190	1,012	91	137	162	194	4,649	493	540	695	810
	23R	976	66	152	168	146	987	58	144	114	124	985	70	104	126	139	4,524	453	589	762	742
	103	1,008	72	92	157	178	987	68	58	94	114	1,012	52	94	120	159	4,562	211	396	509	569
	103	976	114	152	143	137	957	64	69	84	113	985	40	93	129	160	4,524	289	415	415	570
III	96+	1,008	13	29	53	66	957	28	33	24	33	1,012	30	42	42	49	4,649	444	636	948	985
	96+	842	25	43	28	56	987	20	58	54	68	970	25	49	34	28	4,524	259	406	667	712
	1Z	1,008	16	44	101	86	1,083	9	53	67	145	1,012	52	76	91	86	4,524	163	368	279	319
	1Z	976	21	66	65	75	957	33	38	52	60	970	52	62	82	62	4,562	125	288	406	491
IV	98C	1,008	26	53	72	92	987	20	49	49	39	1,012	60	67	79	79	4,649	135	193	231	212
	98C	842	15	34	49	34	957	33	38	64	84	970	44	34	55	82	4,524	135	211	192	230
	132	842	25	28	46	56	1,083	18	43	48	67	970	25	52	82	66	4,562	163	259	396	317
	132	976	34	60	63	60	987	34	68	49	86	985	33	12	59	70	4,524	173	221	279	378
V	H1	1,008	13	29	44	50	1,083	33	126	96	86	1,012	46	86	171	140	4,524	96	163	299	240
	H1	842	15	34	28	34	987	20	54	34	68	970	21	75	52	75	4,649	96	115	250	221
	H19	842	18	25	46	34	957	28	64	80	84	970	52	66	55	89	4,649	39	77	154	202
	H19	976	15	28	25	21	957	38	56	69	60	985	49	90	81	113	4,562	28	57	125	134

TABLE 14.—CHANGES IN MILLIGRAMS PER 100 CC IN THE AMINO-ACID NITROGEN CONTENT (FORMOL TITRATION) OF SOLUTIONS IN WHICH THE FIVE TYPES OF HEAT-RESISTANT BACTERIA ARE GROWING.

TYPE No.	CULTURE No.	D-GLUCOSE BROTH				LACTOSE BROTH				SUCROSE BROTH				SKIMMILK			
		0 days	7 days	15 days	30 days	0 days	7 days	15 days	30 days	0 days	7 days	15 days	30 days	0 days	7 days	15 days	30 days
I	1G	13.41	15.76	13.66	14.22	13.41	21.84	14.11	——	13.41	13.55	13.78	14.90	10.07	11.20	14.11	12.99
	98A	13.41	12.99	12.77	13.55	13.41	14.90	15.34	14.00	13.41	19.26	16.80	22.06	10.07	9.63	12.77	15.01
	52G	——	——	——	——	——	——	——	——	——	——	——	——	10.07	14.56	13.22	11.20
	130	——	——	——	——	——	——	——	——	——	——	——	——	10.07	——	12.54	19.04
	136	——	——	——	——	——	——	——	——	——	——	——	——	10.07	16.35	13.22	12.32
II	23R	11.61	13.22	21.95	18.82	11.61	13.89	17.70	18.27	11.61	17.25	——	19.82	10.07	15.68	13.22	10.75
	103	11.61	13.89	14.22	14.11	11.61	18.26	16.13	17.14	11.61	19.15	19.15	18.82	10.07	13.89	11.42	6.05
III	96+	11.61	20.16	18.51	12.10	11.61	13.66	——	15.90	11.61	10.64	14.78	18.59	10.07	12.54	13.44	15.46
	1Z	16.02	24.42	25.76	33.15	16.02	30.35	31.81	31.14	16.02	28.78	32.37	31.36	10.07	19.26	15.23	——
IV	98C	11.41	14.22	15.79	16.46	11.41	14.90	17.47	16.35	11.41	16.80	14.56	17.14	10.07	——	11.87	15.23
	132	13.41	17.58	——	10.53	13.41	14.34	——	14.45	13.41	13.66	——	14.56	10.07	9.86	17.47	12.99
V	H1	16.02	29.57	30.58	33.38	16.02	30.80	31.36	33.15	16.02	28.56	26.10	32.93	10.07	15.23	14.11	15.01
	H19	13.41	12.43	15.46	16.02	13.41	16.35	16.24	15.90	13.41	18.59	20.50	18.93	10.07	14.56	13.66	17.92

TABLE 15.—CHANGES IN MILLIGRAMS PER 100 CC IN THE AMMONIA NITROGEN CONTENT OF SOLUTIONS IN WHICH THE FIVE TYPES OF HEAT-RESISTANT SPECIES ARE GROWING.

Type No.	Culture No.	Plain broth		D-glucose broth					Lactose broth					Sucrose broth					Skimmilk				
		0 days	7 days	0 days	3 days	7 days	15 days	30 days	0 days	3 days	7 days	15 days	30 days	0 days	3 days	7 days	15 days	30 days	0 days	3 days	7 days	15 days	30 days
I	1G	1.40	1.68	1.26	5.04	3.92	3.08	4.48	1.26	5.04	3.92	3.92	4.20	1.26	2.80	3.08	2.80	3.36	2.12	6.16	7.00	7.84	6.16
	1G	1.26	2.52	1.26	4.48	4.48	3.92	3.64	1.26	5.04	4.48	4.48	4.76	1.26	3.64	4.20	3.08	2.80	2.12	7.84	6.44	5.88	7.00
	98A	1.26	1.96	1.26	4.20	3.36	3.36	3.64	1.26	3.64	3.36	3.64	3.92	1.26	3.92	3.92	4.20	4.76	2.12	5.04	7.56	6.16	6.72
	98A	1.26	1.96	1.26	4.76	4.20	3.92	4.48	1.26	4.20	3.92	4.48	4.48	1.26	4.48	3.64	4.48	4.20	2.12	5.60	5.88	7.28	5.60
	52G	1.26	1.96	——	——	——	——	——	——	——	——	——	——	——	——	——	——	——	2.12	7.56	6.72	5.88	6.44
	52G	1.26	2.24	——	——	——	——	——	——	——	——	——	——	——	——	——	——	——	2.12	5.32	6.16	7.00	6.44
	130	1.26	1.12	——	——	——	——	——	——	——	——	——	——	——	——	——	——	——	2.12	6.16	7.84	7.00	5.60
	130	1.26	1.40	——	——	——	——	——	——	——	——	——	——	——	——	——	——	——	2.12	5.60	6.72	6.44	6.72
	136	1.26	2.52	——	——	——	——	——	——	——	——	——	——	——	——	——	——	——	2.12	6.16	7.28	7.56	6.16
	136	1.26	1.68	——	——	——	——	——	——	——	——	——	——	——	——	——	——	——	2.12	7.28	5.60	8.12	7.28
II	23R	1.26	3.08	1.26	4.20	3.92	5.32	3.92	1.26	5.88	3.92	5.04	6.44	1.26	3.08	3.64	4.20	3.92	2.12	3.08	3.64	3.64	4.76
	23R	1.26	2.24	1.26	4.76	5.04	4.48	4.20	1.26	5.60	4.48	5.88	7.00	1.26	5.32	4.48	3.64	4.20	2.12	3.08	3.08	4.20	5.60
	103	1.26	1.12	1.26	5.18	4.48	5.04	4.48	1.26	5.04	5.88	4.48	4.76	1.26	3.92	4.76	4.76	5.60	2.12	3.36	3.92	3.64	4.76
	103	1.26	1.26	1.26	4.20	4.76	4.48	4.76	1.26	5.60	4.76	5.32	5.80	1.26	5.60	5.60	5.32	5.32	2.12	3.08	3.64	3.08	3.36
III	96+	1.26	3.92	1.26	5.04	2.80	3.36	2.80	1.26	4.76	3.36	4.20	4.20	1.26	3.08	3.08	3.36	3.36	2.12	7.28	8.96	11.20	10.08
	96+	1.26	2.80	1.26	3.64	3.36	4.76	3.92	1.26	5.04	3.92	4.76	5.04	1.26	2.52	3.64	3.92	3.64	2.12	8.96	11.20	11.20	11.20
	1Z	1.26	4.48	1.26	3.64	2.52	3.36	3.64	1.26	4.76	2.80	2.80	5.04	1.26	4.48	4.76	3.92	3.76	2.12	5.32	7.28	9.52	9.52
	1Z	1.26	5.32	1.26	4.48	3.64	4.48	4.76	1.26	4.76	4.20	3.36	4.48	1.26	3.92	3.64	3.36	4.20	2.12	7.56	8.12	8.96	10.36
IV	98C	1.26	3.36	1.26	3.92	1.96	4.48	3.92	1.26	3.92	3.36	4.20	3.36	1.26	4.76	3.92	4.20	3.64	2.12	8.40	8.68	8.68	9.24
	98C	1.26	2.52	1.26	4.20	2.24	5.04	4.76	1.26	3.92	3.92	3.08	4.76	1.26	4.20	3.64	3.08	3.08	2.12	6.72	8.40	9.52	10.36
	132	1.26	4.48	1.26	2.80	3.08	2.80	3.92	——	——	——	——	——	——	——	——	——	——	2.12	5.32	6.16	7.84	8.40
	132	1.40	3.36	1.26	2.24	4.76	3.36	3.08	——	——	——	——	——	——	——	——	——	——	2.12	6.44	7.84	8.96	8.40
V	H1	1.40	5.04	1.26	4.20	4.48	5.60	4.48	1.26	5.32	5.04	6.44	4.76	1.26	4.76	5.04	5.04	5.32	2.12	4.48	5.60	3.92	4.48
	H1	1.40	3.92	1.26	3.08	3.92	5.60	4.76	1.26	4.48	5.60	5.60	3.36	1.26	4.20	4.48	5.60	5.60	2.12	6.72	5.32	5.32	5.04
	H19	1.26	3.64	1.26	2.80	3.64	4.48	4.48	1.26	3.08	4.48	4.48	4.76	1.26	5.32	4.20	4.48	5.04	2.12	5.04	6.16	5.60	4.76
	H19	1.26	4.20	1.26	3.64	3.08	5.04	4.76	1.26	4.48	3.64	4.20	3.64	1.26	4.76	5.04	5.04	4.76	2.12	5.88	5.60	4.76	5.88

TABLE 16.—CHANGES IN GRAMS PER 100 CC IN THE TITRATABLE ACIDITY OF SOLUTIONS IN WHICH THE FIVE SPECIES OF HEAT-RESISTANT ORGANISMS ARE GROWING.

TYPE NO.	CULTURE NO.	D-GLUCOSE BROTH					LACTOSE BROTH					SUCROSE BROTH					SKIMMILK				
		0 days	3 days	7 days	15 days	30 days	0 days	3 days	7 days	15 days	30 days	0 days	3 days	7 days	15 days	30 days	0 days	3 days	7 days	15 days	30 days
I	1G	0.030	0.050	0.070	0.095	0.105	0.030	0.070	0.075	0.100	0.130	0.030	0.055	0.065	0.070	0.090	0.165	0.280	0.325	0.330	0.360
	1G	0.030	0.060	0.085	0.120	0.090	0.030	0.045	0.060	0.105	0.150	0.030	0.045	0.055	0.035	0.065	0.165	0.210	0.240	0.280	0.340
	98A	0.030	0.080	0.095	0.105	0.110	0.030	0.100	0.105	0.130	0.145	0.030	0.070	0.095	0.110	0.125	0.165	0.220	0.270	0.330	0.350
	98A	0.030	0.095	0.080	0.090	0.100	0.030	0.110	0.115	0.130	0.160	0.030	0.100	0.095	0.115	0.115	0.165	0.260	0.280	0.315	0.330
	52G	——	——	——	——	——	——	——	——	——	——	——	——	——	——	——	0.165	0.270	0.295	0.310	0.345
	52G	——	——	——	——	——	——	——	——	——	——	——	——	——	——	——	0.165	0.250	0.270	0.300	0.325
	130	——	——	——	——	——	——	——	——	——	——	——	——	——	——	——	0.165	0.300	0.310	0.325	0.350
	130	——	——	——	——	——	——	——	——	——	——	——	——	——	——	——	0.165	0.240	0.275	0.300	0.345
	136	——	——	——	——	——	——	——	——	——	——	——	——	——	——	——	0.165	0.295	0.310	0.325	0.340
	136	——	——	——	——	——	——	——	——	——	——	——	——	——	——	——	0.165	0.325	0.350	0.380	0.375
II	23R	0.030	0.115	0.160	0.205	0.220	0.030	0.190	0.185	0.160	0.170	0.030	0.220	0.240	0.270	0.275	0.165	0.610	0.660	0.680	0.750
	23R	0.030	0.150	0.200	0.230	0.260	0.030	0.175	0.160	0.125	0.170	0.030	0.190	0.200	0.275	0.250	0.165	0.720	0.740	0.780	0.805
	103	0.303	0.140	0.175	0.190	0.230	0.030	0.160	0.165	0.170	0.180	0.030	0.200	0.215	0.240	0.285	0.165	0.700	0.720	0.740	0.775
	103	0.030	0.160	0.225	0.235	0.250	0.030	0.210	0.180	0.190	0.200	0.030	0.225	0.215	0.255	0.270	0.165	0.680	0.710	0.735	0.760
III	96+	0.030	0.090	0.110	0.130	0.170	0.030	0.055	0.075	0.090	0.110	0.030	0.045	0.055	0.070	0.110	0.165	0.400	0.460	0.495	0.530
	96+	0.030	0.085	0.085	0.145	0.160	0.030	0.065	0.100	0.110	0.135	0.030	0.065	0.070	0.125	0.145	0.165	0.420	0.580	0.560	0.530
	1Z	0.030	0.120	0.150	0.160	0.185	0.030	0.110	0.145	0.140	0.170	0.030	0.065	0.070	0.090	0.110	0.165	0.340	0.390	0.440	0.500
	1Z	0.030	0.145	0.180	0.200	0.230	0.030	0.130	0.155	0.160	0.185	0.030	0.110	0.155	0.170	0.175	0.165	0.425	0.440	0.495	0.510
IV	98C	0.030	0.125	0.120	0.140	0.160	0.030	0.090	0.100	0.120	0.135	0.030	0.080	0.120	0.125	0.140	0.165	0.410	0.425	0.450	0.470
	98C	0.030	0.080	0.150	0.090	0.105	0.030	0.095	0.125	0.120	0.145	0.030	0.045	0.085	0.110	0.125	0.165	0.400	0.410	0.450	0.485
	132	0.030	0.090	0.115	0.140	0.170	——	——	——	——	——	——	——	——	——	——	0.165	0.400	0.420	0.455	0.490
	132	0.030	0.130	0.130	0.165	0.170	——	——	——	——	——	——	——	——	——	——	0.165	0.440	0.435	0.445	0.510
V	H1	0.030	0.100	0.125	0.140	0.155	0.030	0.090	0.100	0.100	0.110	0.030	0.080	0.090	0.115	0.130	0.165	0.300	0.310	0.335	0.355
	H1	0.030	0.125	0.140	0.160	0.175	0.030	0.110	0.145	0.165	0.165	0.030	0.100	0.120	0.155	0.155	0.165	0.260	0.275	0.300	0.330
	H19	0.030	0.090	0.100	0.110	0.115	0.030	0.085	0.090	0.110	0.125	0.030	0.075	0.095	0.110	0.130	0.165	0.250	0.270	0.295	0.310
	H19	0.030	0.085	0.090	0.095	0.101	0.030	0.080	0.085	0.120	0.140	0.030	0.100	0.105	0.110	0.125	0.165	0.325	0.325	0.325	0.350

TABLE 17.—VOLATILE ACID CONTENT OF SOLUTIONS IN WHICH THE FIVE SPECIES OF HEAT-RESISTANT ORGANISMS ARE GROWING.
(Number of cc of N/10 NaOH to neutralize volatile acid in 100 cc of solution.)

TYPE No.	CULTURE No.	PLAIN BROTH			D-GLUCOSE BROTH			LACTOSE BROTH			SUCROSE BROTH			SKIMMILK		
		Uninoculated	Culture 10 to 14 days old		Uninoculated	Culture 10 to 14 days old		Uninoculated	Culture 10 to 14 days old		Uninoculated	Culture 10 to 14 days old		Uninoculated	Culture 10 to 14 days old	
			Without $CaCO_3$	With $CaCO_3$		Without $CaCO_3$	With $CaCO_3$		Without $CaCO_3$	With $CaCO_3$		Without $CaCO_3$	With $CaCO_3$		Without $CaCO_3$	With $CaCO_3$
I	1G	1.6	1.9	1.8	1.6	2.4	2.5	1.6	—	3.6	1.6	1.4	3.3	2.0	2.5	—
	1G	1.6	1.7	1.8	1.6	2.9	.21	1.6	—	3.1	1.6	1.4	3.6	2.0	3.3	—
	98A	1.6	1.8	1.7	1.6	2.8	—	1.6	2.5	—	1.6	2.4	—	2.0	5.3	6.3
	98A	1.6	1.4	1.8	1.6	2.4	—	1.6	2.3	—	1.6	2.5	—	2.0	4.5	4.8
	52G	1.6	1.6	—	1.6	3.2	—	1.6	3.7	—	1.6	1.6	—	—	—	—
	52G	1.6	1.7	—	1.6	2.6	—	1.6	2.5	—	1.6	1.8	—	—	—	—
	130	1.6	1.0	—	—	—	—	—	—	—	—	—	—	2.0	4.2	—
	130	1.6	1.2	—	—	—	—	—	—	—	—	—	—	2.0	3.0	—
	136	1.6	1.7	1.8	1.6	2.5	—	—	—	—	—	—	—	2.0	3.7	—
	136	1.6	1.6	1.8	1.6	2.4	—	—	—	—	—	—	—	2.0	3.2	—
II	23R	1.6	1.9	1.8	1.6	2.4	2.5	1.6	3.3	3.0	1.6	2.1	3.6	2.0	3.6	4.8
	23R	1.7	1.9	1.6	1.6	2.0	2.6	1.6	1.9	2.2	1.6	2.1	2.9	2.0	4.5	5.1
	103	1.6	2.1	2.2	1.6	1.7	—	1.6	1.6	—	1.6	1.6	—	2.0	3.4	4.2
	103	1.6	2.3	2.0	1.6	2.1	—	1.6	1.9	—	1.6	1.7	—	2.0	3.9	4.1
III	96+	1.6	2.4	2.8	1.6	2.4	—	1.6	2.0	—	1.6	2.3	—	2.0	8.6	—
	96+	1.6	2.7	2.4	1.6	2.2	—	1.6	2.2	—	1.6	1.7	—	2.0	7.3	—
	1Z	1.6	2.0	2.6	1.6	1.5	3.9	1.6	1.9	3.7	1.6	1.9	3.9	2.0	5.0	—
	1Z	1.6	2.2	2.4	1.6	1.9	3.4	1.6	2.0	3.8	1.6	2.0	4.1	2.0	4.6	—
IV	98C	1.7	3.1	3.0	1.6	4.8	13.2	1.6	4.5	17.3	1.6	4.2	13.3	2.0	17.0	19.2
	98C	1.6	2.6	2.8	1.6	4.5	12.0	1.6	4.0	13.4	1.6	4.1	12.5	2.0	14.0	16.7
	132	1.6	3.4	3.0	1.6	7.0	—	1.6	5.5	—	1.6	6.1	—	3.0	21.0	—
	132	1.6	3.6	2.6	1.6	8.4	—	1.6	5.4	—	1.6	4.9	—	2.0	18.0	—
V	H1	1.6	1.4	1.8	1.6	1.4	2.4	1.6	1.6	1.5	1.6	1.6	1.5	3.0	4.1	—
	H1	1.6	1.5	1.8	1.6	1.6	2.8	1.6	1.5	2.0	1.6	1.7	2.0	2.0	4.2	—
	H19	1.6	1.6	1.9	1.6	1.9	—	1.6	1.7	—	1.6	1.6	—	2.0	4.1	3.1
	H19	1.6	1.6	1.8	1.6	1.8	—	1.6	1.7	—	1.6	1.6	—	2.0	4.3	2.6

the tests, the cultures were grown in sterile litmus milk for several generations in order to increase their vitality.

Whether freshly isolated cultures would grow at pasteurizing temperatures in raw milk has not been determined. Ayers and Johnson's rod is reported to be larger than the form which appears in the observed laboratory strains.

TYPE II—*Streptococcus thermophilus* ORLA-JENSEN, 1919

Orla-Jensen (1919) found that the commonest lactic acid bacterium, *Streptococcus lactis* (Lister) Löhnis, was killed at 70°C in 15 minutes. This species usually coagulates milk in less than 24 hours at temperatures ranging between 25° and 37°C. *Streptococcus mastiditis* Migula is killed at 60°C in 15 minutes (Orla-Jensen, 1919). Ayers and Johnson (1918) found that 27 strains of pathogenic streptococci were killed in 30 minutes at 140°F when heated in milk. It is evident that Type II is more resistant to heat than the above-mentioned species.

Orla-Jensen described a *Streptococcus thermophilus* which survived in large numbers a temperature of 70°C for 15 minutes but was nearly all killed when exposed at 75°C for 15 minutes. Patzschke (1916) described a *Streptococcus lactis thermophilus* which was killed at 85°C in 10 to 20 minutes, at 80°C in 30 minutes, at 75°C in from 1 to 3 hours, and at 70°C in 5 hours. This species is probably identical with the one described by Orla-Jensen. However, Orla-Jensen's name, being a binomial, is preferred to that suggested by Patzschke. Hammer and Baker (1926) recognize a variety of the species *Streptococcus lactis* which they call *Streptococcus thermophilus* Orla-Jensen.

TYPE III—*Sarcina lutea* SCHRÖTER, 1872

This species has been isolated much more frequently than any of the others and is recognized by its peculiar heat resistance and its luxuriant bright yellow growth. In liquid media both a tenacious yellow surface pellicle and abundant flocculent sediment appear. Altho similar species have been associated with sterilized milk, Dyar (1895) first described a yellow micrococcus to which he gave the name *Merismopedia flava varians*. Russell and Hastings (1902b) describe it as a micrococcus, the thermal death point of which is 76°C. Ayers and Johnson (1910, 1913) probably saw it among their acid-forming and alkali-forming species which survive the pasteurization of milk. Orla-Jensen (1919) uses the generic term *Tetracoccus*

for organisms of this type. Fenger, Cram, and Rudnick (1920) found it surviving in supposedly sterile surgical sutures and ligatures. More recently, Brannon and Prucha (1927) have observed the unusual heat resistance of this species. The first description of this species was by Schröter (1872) and it is now recognized as *Sarcina lutea*. According to Omeliansky (1911) this species has been isolated from a mastodon carcass in Siberia which had been covered for centuries with ice.

TYPE IV—*Micrococcus conglomeratus* MIGULA, 1900

The heat resistance of this species is less than that of any of the other five. Ayers and Johnson (1910, 1913), no doubt, encountered it among the bacteria which survive pasteurization. Orla-Jensen uses the generic term *Tetracoccus* for organisms of this type. Hucker (1924) has recognized it as *Micrococcus conglomeratus* Migula.

TYPE V—*Sarcina rosea* SCHRÖTER, 1872

The isolation of this species is somewhat rare and its heat resistance is usually not recognized. Fenger, Cram, and Rudnick (1920) isolated it in connection with heat resistance studies. Altho this species is a typical sarcina, the description by Frankland (1887) of *Micrococcus rosaceus* approaches that of this species as closely as any with the exception of the lack of regular packet formation among the cells. Conn, Esten, and Stocking (1906) describe *Micrococcus lactis rosaeus*, a similar species.

DESCRIPTION OF SPECIES

Type I. *Microbacterium lacticum* Orla-Jensen, 1919.

Form: Rod, 0.3 to 0.5 by 0.6 to 1.2 microns, occurs singly, in pairs or small irregular clumps of from 4 to 16 individuals, non-motile, Gram-positive.
Spores: Absent.
Agar colonies: Dull grey or white, opaque, raised centers (when not on crowded plates), round, glistening, slow in developing.
Gelatin liquefaction: Negative.
Nitrate reduction: Usually negative.
Litmus milk: Acid only, not coagulated except in old cultures in the presence of heat.
Indole formation: Negative.
Hydrogen sulfide formation: Negative.
Oxygen relations: Aerobe.
Bouillon: Fine sediment.
Carbohydrates: Usually fermented with acid formation. (Details given in Table 12.)
Volatile acid formation: Negative.

Ammonia nitrogen formation: Negative.
Amino acid nitrogen formation: Negative.
Optimum hydrogen-ion concentration: pH 7.0 to 7.2.
Optimum growth: At ?°C. Laboratory strains grow well. At 30° to 35°C.
Thermal death point: Varies between 80° to 83°C when a 15-hour-old milk culture is heated for 30 minutes. Majority thermal death point is above 74°C for 30 minutes and relatively few, if any, cells are killed at pasteurizing temperatures in milk.

Type II. *Streptococcus thermophilus* Orla-Jensen, 1919.
Form: Coccus, 0.7 to 0.9 micron pointed ends, arranged in pairs usually and sometimes chains of 4 to 6 cells.
Spores: Absent.
Agar colonies: Small, round and white, like those of *Streptococcus lactis* (Lister) Löhnis.
Agar slant: Scanty, bead-like, white.
Gelatin liquefaction: Negative.
Nitrate reduction: Negative.
Litmus milk: Acid and coagulated by freshly isolated cultures in two to four days. Litmus partly reduced sometimes.
Indole formation: Negative.
Hydrogen sulfide formation: Negative.
Oxygen relations: Aerobe.
Bouillon: Fine sediment.
Carbohydrates: Usually fermented with acid formation. (Details given in Table 12.)
Volatile acid formation: Negative.
Ammonia nitrogen formation: Negative.
Amino acid nitrogen formation: Negative.
Optimum growth: At 40°C.
Thermal death point: Varies between 72° and 74°C in milk culture 15 hours old when heated for 30 minutes. Majority thermal death point is above 65°C in milk for 30 minutes.

Type III. *Sarcina lutea* Schröter, 1872.
Form: Coccus, 1.2 to 1.5 microns in diameter, arranged like a typical sarcina in cubical packets, non-motile, Gram-positive.
Spores: Absent.
Agar colonies: Medium sized, smooth, circular, centers raised, yellow.
Agar slant: Smooth, raised, abundant, yellowish growth.
Gelatin liquefaction: Positive.
Nitrate reduction: Usually positive.
Litmus milk: Acid, coagulated in 4 to 6 days when freshly isolated. Litmus usually partly reduced.
Indole: Usually negative.
Hydrogen sulfide production: Usually negative.
Oxygen relations: Aerobe.
Bouillon: Abundant yellow flocculent sediment. Frequently a surface pellicle develops.

Carbohydrates: Not readily fermented. Medium usually becomes alkaline. (Details given in Table 12.)
Volatile acid formation: Slight.
Ammonia nitrogen formation: Negative.
Amino acid nitrogen formation: Slight increase.
Optimum hydrogen-ion concentration: pH 7.0.
Optimum growth: At 30° to 37°C.
Thermal death point: Varies between 74° and 76°C when a 15-hour-old milk culture is heated for 30 minutes. The majority thermal death point is above 68°C for 30 minutes in milk.

Type IV. *Micrococcus conglomeratus* Migula, 1900.
Form: Coccus 0.8 to 1.1 microns in diameter, arranged in irregular mass formation, non-motile, Gram-positive.
Spores: Absent.
Agar colonies: Moderate, smooth, circular, yellow. Not as abundant growth as in Type III.
Agar slant: Moderate, smooth, yellow.
Gelatin liquefaction: Positive.
Nitrate reduction: Positive.
Litmus milk: Acid production only.
Indole formation: Negative.
Hydrogen sulfide formation: Negative.
Oxygen relations: Aerobe.
Bouillon: Abundant yellow sediment.
Carbohydrates: Usually fermented with acid formation. (Details given in Table 12.)
Volatile acid formation: Positive.
Ammonia nitrogen formation: Negative.
Amino acid nitrogen formation: Negative.
Optimum hydrogen-ion concentration: pH 7.0.
Optimum growth: 30° to 37°C.
Thermal death point: Varies from 66° to 68°C when a milk culture 15 hours old is heated for 30 minutes. The majority thermal death point is above 63°C. This applies to the two cultures under observation and not to the species as a whole.

Type V. *Sarcina rosea* Schröter, 1872.
Form: Coccus, 1.2 to 1.6 microns in diameter, arranged like typical sarcinae in cubical packets, non-motile, Gram-positive.
Spores: Absent.
Agar colonies: Medium sized, round, smooth, raised in centers, pink to flesh colored.
Agar slant: Moderate, smooth, raised, flesh colored. Growth is slow.
Gelatin liquefaction: Positive.
Nitrate reduction: Usually positive.
Litmus milk: Slight acid formation.
Indole formation: Negative.
Hydrogen sulfide formation: Negative.
Oxygen relations: Aerobe.
Bouillon: Slightly turbid with flesh colored sediment.

Carbohydrates: Not readily fermented. Medium frequently becomes alkaline. (Details given in Table 12.)
Volatile acid formation: Negative.
Ammonia nitrogen formation: Negative.
Amino acid nitrogen formation: Negative or very slight increase.
Optimum growth: 30° to 37°C.
Thermal death point: Varies between 70° and 73°C when a milk culture 15 hours old is heated for 30 minutes. The majority thermal death point is above 67°C when heated for 30 minutes in milk.

SUMMARY AND CONCLUSIONS

A series of pasteurized milk plates from various cities in this country were sent to the New York State Agricultural Experiment Station for the purpose of studying the so-called "pin-point" flora of pasteurized milk. A few heat-resistant cultures were also sent to the laboratory. A series of heat-resistant cultures were isolated by the author using enrichment methods. The methods consisted of holding samples of raw milk at pasteurizing temperatures and plating hourly for seven hours. A further selection of cultures was made by determining the percentage reduction in the plate count after pasteurization. Where there was more than 90 per cent survival the cultures were retained for further study.

These cultures were divided morphologically into five types, which proved to belong to five distinct species. The utilization of various carbohydrates, some of the higher alcohols, organic acids, and amino acids was determined. Quantitative determinations of volatile acids from milk, d-glucose, lactose, and sucrose were made at the end of two weeks. The amount of ammonia and amino-acid nitrogen and the titratable acid were determined at various times up to 30 days.

Three species, *Microbacterium lacticum* Orla-Jensen (1919), *Streptococcus thermophilus* Orla-Jensen (1919), and *Sarcina lutea* Schröter (1872) were common in milk. Of these three, *Sarcina lutea* could be isolated most frequently. *Micrococcus conglomeratus* Migula (1900) was isolated twice and *Sarcina rosea* Schröter (1872) once. *Microbacterium lacticum* has been developed by enrichment methods in 5 out of 60 samples from individual producers of milk.

The "majority" thermal death points of the above species was above the customary pasteurizing temperatures for milk. The decreasing order of the thermal resistance of the species is as follows: *Microbacterium lacticum, Sarcina lutea, Streptococcus thermophilus, Sarcina rosea,* and *Micrococcus conglomeratus.*

The presence of the above species in market milk as indicated by the fermentative studies is not regarded as dangerous to health. Their rates of growth, with the exception of *Streptococcus thermophilus*, are relatively slow at temperatures ranging between 20° and 37°C.

LITERATURE CITED

Adametz, L.

1890 Die Bakterien normaler und abnormaler Milch. *Milchztg.*, **19,** *255–256.*

Adams, F. O., and Harding, H. A.

1924 Observations on thermophilic bacteria in milk from farms. *Abst. Bact.*, **8,** *18.*

Adelloff, A. von

1907 Etwas über die Lebensdauer der Milchsäuerbakterien. *Milchwirtsch. Zentralbl.*, **3,** *233-235.*

Ayers, S. H., and Johnson, Jr., W. T.

1910 The bacteriology of commercially pasteurized and raw market milk. *United States Dept. Agr. Bur. Animal Ind. Bul. No. 126.*

1913 A study of the bacteria which survive pasteurization. *United States Dept. Agr. Bur. Animal Ind. Bul. No. 161.*

1914 Ability of streptococci to survive pasteurization. *Jour. Agr. Res.*, **2,** *321–330.*

1915 Ability of colon bacilli to survive pasteurization. *Jour. Agr. Res.*, **3,** *401–410.*

1923 The problem of transporting milk in hot condition. *Jour. Dairy Sci.*, **6,** *608–615.*

1924a Studies on pasteurization. XI. The "majority" and "absolute" thermal death points of bacteria in relation to pasteurization. *Jour. Bact.*, **9,** *279–284.*

1924b Studies on pasteurization. XII. Cause and significance of pin-point colonies from pasteurized milk. *Jour. Bact.*, **9,** *285–300.*

Ayers, S. H., Johnson, Jr., W. T., and Davis, B. J.

1918 The thermal death point and limiting pH of pathogenic streptococci. *Jour. Inf. Dis.*, **23,** *290–300.*

Behrens, M.

1923 Über die Bedingungen der Widerstandsfähigkeit von Bakterien gegen Erhitzung. *Ztschr. f. Hyg.*, **100,** *388–401.*

Bertrand, G.
1906 Le dosage des sucres réducteurs. *Bull. Soc. Chim. d. France*, **35,** *1285–1299.*

Bleyer, L.
1923 Der Nachweis der Abtötung durch Kultur und Tierversuch bei mit Hitze behandelten Bakterien. *Ztschr. f. Hyg.*, **99,** *98–100.*

Brannon, J. M., and Prucha, M. J.
1927 The effect of the pasteurization temperature on individual germs found in milk. *Jour. Dairy Sci.*, **10,** *263-268.*

Brown, C. W., and Peiser, K.
1916 Keeping qualities of butter. V. Pasteurization and its influence. A study of the factors which influence the resistance of lactic acid bacteria to heat. *Michigan Agr. Exp. Sta. Tech. Bul. No. 30.*

Brown, J. H.
1923 The formol titration of bacteriological media. *Jour. Bact.*, **8,** *245–267.*

Clarke, J. J., and Dougherty, J. M
1926 A study of the thermophilic bacteria in pasteurized milk. *Doctor's thesis, Catholic University of America, Washington, D. C.*

Conn, H. W., Esten, W. M., and Stocking, Jr., W. A.
1906 A classification of dairy bacteria. *Connecticut (Storrs) Agr. Exp. Sta., 18th Ann. Rpt., 91–203.*

Cooledge, L. H.
1924 A probable explanation of high counts (pin-point colonies) in pasteurized milk. *Abst. Bact.*, **8,** *20.*

Dotterrer, W. D.
1923 Some observations on high counts in milk freshly pasteurized under commercial conditions. *Internat. Assoc. Dairy and Milk Inspectors, 12th Ann. Rpt., 204–214.*

Downs, P. A.
1925 A study of the organisms causing thickening of sweetened condensed milk. *Jour. Dairy Sci.*, **8,** *344–369.*

Dyar, H. G.
1895 On certain bacteria from the air of New York City. *Ann. New York Acad. Sci.*, **8,** *322–380.*

Eijkmann, C.
1908 Die Überlebungskurve bei Abtötung von Bakterien durch

Hitze. *Biochem. Ztschr.*, **11**, *12–20*. Abstract in *Centralbl. f. Bakteriol., II Abt.*, **22**, *508*.

Fay, A. C.

1926 Thermotolerant saccharophilic organisms as a cause of "pin-point" colonies in the bacteriological analysis of ice cream. *Jour. Bact.*, **11**, *96–97*.

1927 Thermo-tolerant organisms as a cause of so-called pin-point colonies. *Jour. Bact.*, **13**, *347-377*.

Fenger, F., Cram, E. B., and Rudnick, P.

1920 Heat resistant organisms. A study of the bacteria encountered in heat sterilization of surgical ligatures and sutures. *Jour. Amer. Med. Assoc.*, **74**, *24–25*.

Fleischer, L., and Amster, S.

1923 Über den Einfluss des Mediums auf die Resistenz der Bakterien Desinfektionsversuche mit Hitze. *Ztschr. f. Hyg.*, **99**, *209–220*.

Folin, O.

1902 Eine neue Methode zur Bestimmung des Ammoniaks im Harne und andere thierischen Flüssigkeiten. *Ztschr. f. physiol. Chemie*, **37**, *161–176*.

Frankland, P., and Frankland, G.

1887 On some new microorganisms obtained from air. *Philos. Trans. Roy. Soc., London*, **178**, *257–287*.

Freudenreich, E. von, and Thöni, J.

1904 Über die Wirkung verschiedener Milchsäurefermente auf die Kasereifung. *Landwirtsch. Jahrb. d. Schweiz*, **18**, *525–548*.

Gage, S. de M., and Stoughton, G. van E.

1906 A study of the laws governing the resistance of *B. coli* to heat. *Tech. Quart.*, **19**, *41–54*.

Göbel, R.

1923 Über die Beziehungen zwischen der Dickte einer Bakterienaufschwemmung und ihre Widerstandsfähigkeit gegen Erhitzung. *Ztschr. f. Hyg.*, **100**, *380–387*.

Gorini, C.

*1915 [Resistance of non-spore-forming bacteria to the action of heat.] *Rend. R. Institut. Lombardo Sci. Lettere*, **48**, *956–961*. Abstract in *Analyst*, **41**, *381*.

*Seen in abstract only.

1921 Weitere Untersuchungen über die Biologie der Milchsäurebacterien. *Centralbl. f. Bakteriol., II Abt.*, **53**, *284–287*.

Greig-Smith, R.

*1909 The coagulation of condensed milk *Linn. Soc. N. S. Wales, March, p. 8.* Abstract in *Bot. Centralbl.*, **111**, *347*.

Hammer, B. W., and Baker, M. P.

1923 Studies on the *Streptococcus paracitrovorus* group. *Iowa Agr. Exp. Sta. Res. Bul. No. 81.*

1926 Classification of the *Streptococcus lactis* group. *Iowa Agr. Exp. Sta. Res. Bul. No. 99.*

Harding, H. A.

1923 Sources of variation in bacterial count of pasteurized milk. *Abst. Bact.*, **7**, *23-24*.

Harding, H. A., and Ward, A. R.

1924 Thermophilic bacteria in composite samples from milk plants. *Abst. Bact.*, **8**, *18*.

1926 Thermophilic bacteria and milk pasteurization. *Jour. Bact.*, **11**, *95–96*.

Harding, H. G.

1927 The occurrence and significance of thermophilic bacteria in milk. *Doctor's thesis. University of Wisconsin. Madison, Wis.*

Hashimoto, S.

1901 Zwei neue milchsäurebildende Kugelbakterien. *Hyg. Rund.*, **11**, *821–834*.

Hauser

*1887 Entgegnung auf die Bemerkung des Herrn cand. med. Schedtler über die Zoogloeabildung und das Schwärmstadium der Proteusarten. *Münchener med. Wochenschrift*, **34**, *492*.

Henneberg, W.

*1906 Zur Kenntnis der Abtötungstemperature der aus dem Malz lebenden schädlichen Mikroorganismen. *Zeitschr. f. Spiritus-industrie*, **28**, *No. 11*, Abstract in *Centralbl. f. Bakteriol., II Abt.*, **16**, *761–763*.

Holman, W. L.

1914 The relative longevity of different streptococci and possible

*Seen in abstract only.

errors in the isolation and differentiation of streptococci. *Jour. Inf. Dis.*, **15**, *293–308*.

Hucker, G. J

1921 A comparison of the various methods of Gram staining. *Abst. Bact.*, **6**, *2*. Also with Conn, H. J. in *New York Agr. Exp. Sta. Tech. Buls. Nos. 93 and 128*.

1924 Studies on the *Coccaceae*. IV. The classification of the genus *Micrococcus* Cohn. *New York Agr. Exp. Sta. Tech. Bul. No. 102*.

Hungerford, J. D., and Harding, H. A.

1924 Influence of the period of operation of the pasteurizer upon the bacterial count of the milk. *Abst. Bact.*, **8**, *17*.

Jacobsen, G. J.

1918 Unusual factors influencing the efficiency of pasteurization. *Milk Dealer*, **7**, *18–20*. Also *Abst. Bact.*, **2**, *215*.

Jenkins, H.

1926 Experiments on the pasteurization of milk, with reference to the efficiency of commercial pasteurization. *Jour. Hyg.*, **25**, *273–284*.

Johnson, S. R., and Exworthy, A.

1925 Thermophilic streptococci isolated from milk and their relation to pin-point colonies. *Abst. Bact.*, **9**, *24*.

Jong, D. A. de, and Graaff, W. C. de

1906 The Coli-Kontrolle der pasteurisierten Milch. *Nederlandsch. Weekblad voor Zuivelbereiding en Veeteelt*, **12**, *Nos. 37, 38*. Abstract in *Milchwirtsch. Zentralbl.*, **3**, *265–268. 1907*.

Krumweide, C., and Noble, W. C.

1921 On the claim that some typhoid-paratyphoid strains survive the milk pasteurization. *Jour. Inf. Dis.*, **29**, *310–312*.

Levine, M., and Carpenter, D. C.

1923 Gelatin liquefaction by bacteria. *Jour. Bact.*, **8**, *297–306*.

Lingelsheim, W. von

1912 Streptokokken. *Handbuch d. pathogen. Mikroorganismen. 2nd. ed., Kolle and Wassermann*, **4**, *453–512*.

Magoon, C. A., and Culpepper, C. W.

1921 A study of the factors affecting temperature changes in the container during the canning of fruits and vegetables. *United States Dept. Agr. Bul. No. 956*.

Man, C. de
1893 Über die Einwirkung von hohen Temperaturen auf Tuberkelbacillen. *Arch. f. Hyg.*, **18,** *133–179.*
Marshall, C. E.
1897 Pasteurization of milk. *Michigan Agr. Exp. Sta. Bul. No. 147.*
Migula, W.
1900 System der Bakterien. *Jena:* **1,** *368 pp., 1897;* **2,** *1068 pp., 1900.*
Morrison, L. E., and Tanner, F. W.
1922 Studies on the thermophilic bacteria. I. Aerobic thermophilic bacteria from water. *Jour. Bact.*, **7,** *343–366.*
Omeliansky, W.
1910 Bakteriologische Untersuchungen des Sauga-Jurashschen Mammets und des anliegenden Bodens. *Arch. des Sci. Biol., St. Petersburg,* **16,** *355-366.*
Orla-Jensen, S.
1919 The lactic acid bacteria. *Mém. de l'Acad. Roy. d. Sci. et d. Lettres de Danemark, Copenhague. Séct. d. Sci., 8 sér.,* **5** *(No. 2), 81–196, 51 pl.*
1921 La pasteurisation du lait. *Le Lait.*, **1,** *105–112, 177–183.*
Park, W. H.
1927 Thermal death points of pathogenic bacteria in milk. *Amer. Jour. Pub. Health,* **17,** *36–47.*
Patzschke, W.
1916 Über die Widerstandsfähigkeit von Bakterien gegenüber hohen Temperaturen und das Lobecksche Biorisierverfahren. *Ztschr. f. Hyg.*, **81,** *227–256.*
Peiser, K.
1915 Factors influencing the resistance of lactic acid bacteria to pasteurization. *Science,* **42,** *320.*
Robertson, A. H.
1923 A preliminary report on some non-sporulating, heat-resistant organisms in pasteurized milk. *Abst. Bact.*, **7,** *357.*
1924 The bacterial flora of milking machines. *New York State Agr. Exp. Sta. Tech. Bul. No. 105.*
Rogers, L. A.
1905 The bacteria of pasteurized and unpasteurized milk under laboratory conditions. *United States Dept. of Agr. Bur. Animal Ind. Bul. No. 73.*

Rosenau, M. J.

1908 The thermal death points of pathogenic organisms in milk. *United States Pub. Health Service, Hygienic Laboratory, Bul. Nos. 42 and 56.*

Russell, H. L.

1895 A biological study of pasteurized milk and cream under commercial conditions. *Centralbl. f. Bakteriol., II Abt.*, **1**, *741–751.*

Russell, H. L., and Hastings, E. G.

1901 On the increased resistance of bacteria in milk pasteurized in contact with the air. *Wisconsin Agr. Exp. Sta., 18th Ann. Rept., 185–194.* Abstract in *Centralbl. f. Bakteriol., II Abt.*, **8**, *462–469.*

1902a Conditions affecting the thermal death point of bacteria in milk. *Science*, **15**, *361-362.*

1902b A micrococcus, the thermal death limit of which is 76° C. *Centralbl. f. Bakteriol., II Abt.*, **8**, *339–342.*

Schröter, J.

1872 Über einige durch Bakterien gebildete Pigmente. *Beit. z. Biol. d. Pflanzen.*, **1**, *Heft 2, 109–126.*

Smith, T.

1899 Studies on the tubercle bacillus. *Jour. Exp. Med.*, **4**, *217–233.*

Sörensen, S. P. L.

1907 Études enzymatiques. *Compt. rend. des Travaux du Lab. de Carlsberg*, **7**, *1–57.* Also *Biochem. Ztschr.*, **7**, *45–101. 1908.*

Sternberg, G. M.

1887 The thermal death point of pathogenic organisms. *Amer. Jour. Med. Sci.*, **94**, *146–160.*

Stubenrath

*1897 Das Genus Sarcina in morphologischer, biologischer, und pathogenischer Beziehung, mit besonderer Berücksichtigung der Magensarcina. *Habilitationschrift, Wurzburg.*

Swenarton, J. C.

1925 Observations on "pin-point colony" organisms in the Baltimore milk supply. *Abst. Bact.*, **9**, *23-24.*

*Seen in abstract only.

1926 Observations on "pin-point colony" organisms in the Baltimore milk supply. *Jour. Bact.*, **11**, *285–292*.

Tanner, F. W.

1924 Thermophiles from milk. *Abst. Bact.*, **8**, *18*.

Tanner, F. W., and Harding, H. G.

1926a Thermophilic bacteria from milk. *Jour. Bact.*, **11**, *96*.

1926b Thermophilic bacteria from milk. *Centralbl. f. Bakteriol., II Abt.*, **67**, *330–347*.

Taylor, A. R.

1924 Observations on the increase of the bacterial count during the pasteurization process. *Abst. Bact.*, **8**, *17*.

Taylor, G. B.

1924 Some observations on the scientific control of milk plants from the standpoint of bacteria. *Intern. Assoc. Dairy and Milk Inspectors, 13th Ann. Rpt., 287–291*.

Twiss, E. M.

1920 The effect of pasteurizing temperatures on the paratyphoid group. *Jour. Inf. Dis.*, **26**, *165–170*.

Van Geuns, J.

1889 Über das 'Pasteurisieren' von Bakterien. Ein Beitrag zur Biologie der Mikroorganismen. *Arch. f. Hyg.*, **9**, *369–405*.

Van Horn, R. C.

1924 The influence of beef extract on the bacterial counts of raw and pasteurized milk. *Abst. Bact.*, **8**, *16*.

Ward, A. R.

1926 What ails bacterial counts? Heat resisting bacteria. *Dairy Products Merchandising*, **7**, *27–29, 32–36*.

Weigmann, H., Wolff, A., Trensch, M., and Steffen, M.

1916 Über das Verhalten der Milchsäuerbakterien (*Streptococcus lacticus*) bei der Dauererhitzung der Milch auf 60°-63°C. (Modernes Dauerpasteurisierungs-Verfahren). *Centralbl. f. Bakteriol., II Abt.*, **45**, *63–107*.

Weiss, E.

1899 Über drei in gesäuerten Rübenschnitzeln neu aufgefundene Milchsäuerbakterien. *Jahresb. f. Landwirtsch.*, **47**, *141–161*. Also *Inaug. Diss. Göttingen, 1898*.

Wolff, A.

1908 Zur Kenntnis der Veränderungen in der Bakterien-flora der frischen Milch während des sogenannten Incubations-

stadiums. *Centralbl. f. Bakteriol., II Abt.*, **20,** *651–675, 737-780.*

Yates, J. W.

1923 Pin-point colonies in plates from pasteurized milk. *Abst. Bact.*, **7,** *24.*

Yates, J. W., and Glover, J. J.

1923 The pin-point colonies observed in the bacterial examination of milk; their resistance to heat and growth in different media. *Intern. Assoc. Dairy and Milk Inspectors., 12th Ann. Rpt., 252–261.*

Zelenski, T.

1906 Zur Frage der Pasteurisation der Säuglingsmilch. *Jahrb. f. Kinderheilk.*, **63,** *288–307.*